

# World Journal of *Diabetes*

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*World Journal of Diabetes*

Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China

Telephone: +86-10-85381891

Fax: +86-10-85381893

E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)

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## Milestones in the history of diabetes mellitus: The main contributors

Marianna Karamanou, Athanase Protogerou, Gregory Tsoucalas, George Androutsos, Effie Poulakou-Rebelakou

Marianna Karamanou, Gregory Tsoucalas, George Androutsos, Effie Poulakou-Rebelakou, Department of History of Medicine, Medical School, University of Athens, 11527 Athens, Greece

Athanase Protogerou, Department of Pathophysiology, "Laiko" Hospital, Medical School, University of Athens, 11527 Athens, Greece

**Author contributions:** All authors equally contributed to this paper with conception and design of the study, literature review and analysis, drafting and critical revision and editing, and final approval of the final version.

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**Correspondence to:** Marianna Karamanou, MD, PhD, Department of History of Medicine, Medical School, University of Athens, Greece 4 str, Themidos, Kifissia, 11527 Athens, Greece. [mkaramanou@med.uoa.gr](mailto:mkaramanou@med.uoa.gr)  
Telephone: +30-210-7461437  
Fax: +30-210-8235710

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### Abstract

Diabetes mellitus is a group of metabolic diseases involving carbohydrate, lipid, and protein metabolism. It is characterized by persistent hyperglycemia which results from defects in insulin secretion, or action or both. Diabetes mellitus has been known since antiquity. Descriptions have been found in the Egyptian papyri, in ancient Indian and Chinese medical literature, as well as, in the work of ancient Greek and Arab physicians. In the 2<sup>nd</sup> century AD Aretaeus of Cappadocia provided the first accurate description of diabetes, coining the term diabetes, while in 17<sup>th</sup> century Thomas Willis added the term mellitus to the disease, in an attempt to describe the extremely sweet taste of the urine. The important work of the 19<sup>th</sup> century French physiologist Claude Bernard, on the glycogenic action of the liver, paved the way for further progress in the study of the disease. In 1889, Oskar Minkowski and Joseph von Mering performed their famous experiment of removing the pancreas from a dog and producing severe and fatal diabetes. In 1921, Frederick Banting and Charles Best extended Minkowski's and Mering's experiment. They isolated insulin from pancreatic islets and administered to patients suffering from type 1 diabetes, saving thus the lives of millions and inaugurating a new era in diabetes treatment.

**Key words:** History of endocrinology; Metabolic disorder; Diabetes mellitus; Aretaeus of Cappadocia; Insulin

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**Core tip:** Diabetes mellitus has been known since antiquity and despite therapeutic advances it still remains an incurable chronic disease. In our historical article, we attempt to provide the most important steps in the history of diabetes mellitus from antiquity till nowadays. The contribution of leading medical figures



such as Aretaeus of Cappadocia, Thomas Willis, Claude Bernard, Oskar Minkowski, Joseph von Mering, Frederick Banting and Charles Best is mentioned, in an attempt to highlight the development of our current knowledge in diabetes mellitus.

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## INTRODUCTION

Diabetes mellitus is a group of metabolic diseases involving carbohydrate, lipid, and protein metabolism. It is characterized by persistent hyperglycemia, as a result of defects in insulin secretion, insulin action or a combination of both, defective secretion and incorrect action. There are two main types of diabetes mellitus: Type 1 (insulin-dependent), and type 2 (non-insulin-dependent). Type 1 diabetes results by the autoimmune destruction of the  $\beta$ -cells of the pancreatic islets and type 2 diabetes is caused from impaired insulin secretion and resistance to the action of insulin<sup>[1]</sup>. Current epidemiological data reveal that 9% of adults, 18 years of age and older, has diabetes mellitus while it was estimated that in 2012, 1.5 million people died due to the disease. According to the World Health Organization, diabetes will be the 7<sup>th</sup> leading cause of death in 2030<sup>[2-4]</sup>.

The disease has a long history reaching back into antiquity. However, during that period, due to a poor knowledge of anatomy, pathophysiology and lack of diagnostic tools, the disease remained extremely perplexing to physicians.

Nevertheless, physicians in antiquity observed the distinctive features of diabetes and proposed several therapeutic approaches. In Ebers papyrus, dated back to 1500 BC, we may find passages describing patients who suffer from excessive thirst, copious urination and they are treated by plants' extracts. However, according to the Egyptian endocrinologist, historian of medicine and translator of the Ebers papyrus Paul Ghalioungui (1908-1987), the description of a probable diabetes, in Ebers, is regarded as unsatisfactory and probably wrong. In Kahun papyrus (c. 2000 BC) there is just the title of a recipe for the "Treatment of a thirsty woman", but the text is missing<sup>[5]</sup>. So, we may assume that ancient Egyptians could not recognize behind the symptoms of specific disease entity such as diabetes.

Around the 5<sup>th</sup> century BC, the famous Indian surgeon Sushruta, in his work Samhita, identified diabetes, by using the term madhumeha (honey-like urine) and pointed out not only the sweet taste of the urine but also its sticky feeling to the touch and its ability to attract the ants (!). Sushruta further mention

that diabetes affects primarily the rich castes and is related to the excessive food consumption as the rice, cereals and sweets<sup>[6]</sup>.

In ancient China, Chang Chung-Ching (ca. 160-ca. 219), referred to as "the Chinese Hippocrates", described polyuria, polydipsia and loss of weight as symptoms of a specific disease, while in 7<sup>th</sup> century AD Chen Chuan recorded the sweet urine in diabetes mellitus and named the disease Hsiao kho ping mentioning its characteristic symptoms: intense thirst, copious drinking and large amounts of urine which is tasted sweet. In an attempt to treat that disease his colleague Li Hsuan proposed the abstinence from wine, salt and sex<sup>[6]</sup>.

From the 8<sup>th</sup> century onwards, physicians observed the tendency of diabetic patients to develop skin infections as furuncles, rodent ulcers and troubles of the eyesight. In 11<sup>th</sup> century AD, the celebrated Arabo-Islamic physician Avicenna (980-1037) in his textbook El-Kanun (Canon of Medicine) described diabetes and mentioned gangrene and sexual dysfunction as its complication. Years later, the medieval scholar Moises Maimonides (1138-1204) described in detail diabetes, including the symptoms of acidosis<sup>[6]</sup>.

Ancient Egyptians, Indians, Chinese and Arabs tried to describe the clinical signs and symptoms of diabetes mellitus. However, few are the main protagonists in the history of diabetes mellitus who contributed significantly, not only to its diagnosis and treatment but also to the development of our current notions on the disease, paving the way for further study and establishing a new medical sub specialty, diabetology.

## ARETAEUS OF CAPPADOCIA (2<sup>nd</sup> CENTURY AD) AND THE FIRST ACCURATE DESCRIPTION OF DIABETES

Aretaeus, surnamed the Cappadocian, is probably the greatest physician of the Greco-Roman antiquity after Hippocrates, and at least equal of Galen. He was born in Cappadocia, a region in eastern Asia Minor, studied medicine in Alexandria and practiced in Rome probably during the 2<sup>nd</sup> century AD. Aretaeus' medical practice was based on the principals of the Pneumatic school believing not only in the vital role of pneuma (air) but embracing also the theory of the four humors (heat, coldness, moisture, dryness). In his two treatises, *De causis et signis morborum acutorum et diuturnorum* (on the causes and symptoms of acute and chronic diseases) and *De curatione morborum acutorum et diuturnorum* (on the cure of acute and chronic diseases), written in Ionic dialect, Aretaeus impresses us by the vividness and the simplicity of his descriptions. Among others he described, in an accurate way for his time, leprosy, asthma, pneumonia cancer, tetanus, hysteria, epilepsy, gout<sup>[7,8]</sup> (Figure 1).

Before Aretaeus, ancient Greek medical authors such as Rufus of Ephesus (c. 1<sup>st</sup> century AD) and



Figure 1 The distinguished physician Aretaeus of Cappadocia. (Source: Wellcome Library, London).

Galen (130-c.201) were mentioning that diabetes was provoking excessive thirst, polyuria, emaciation of the human body, leading sometimes to death. The symptom of polyuria gives the idea to Galen, who according to his own writings he has seen the disease only twice, to name diabetes diarrhea urinoma (diarrhea of the urine). Later, the term diabetes was introduced into medical nomenclature by Aretaeus. It arises from the Greek verb διαβαίνω (diabaino) which means I pass through and diabetes, the condition that the fluid runs through.

In the following passage of Aretaeus' work, we may admire the clinical presentation and interpretation of diabetes: "Diabetes is a wonderful affection, not very frequent among men... The course is the common one, namely, the kidneys and the bladder; for the patients never stop making water, but the flow is incessant, .... The nature of the disease, then, is chronic, and it takes a long period to form; but the patient is short-lived, if the constitution of the disease be completely established; for the melting is rapid, the death speedy. Moreover, life is disgusting and painful; thirst; excessive drinking, which, however, is disproportionate to the large quantity of urine, for more urine is passed; and one cannot stop them either from drinking or making water. Or if for a time they abstain from drinking, their mouth becomes parched and their body dry; they are affected with nausea, restlessness, and a burning thirst; and at no distant term they expire. Thirst, as if scorched up with fire... But if it increase still more, the heat is small indeed, but pungent, and seated in the intestines; the abdomen shriveled, veins protuberant, general emaciation, when the quantity of urine and the thirst have already increased; and when, at the same time, the sensation appears at the extremity of the member, the patients immediately make water". For the treatment of the disease he proposes the consumption

of cereals, milk and wine, the topical application of cataplasms and the administration of Theriac, the famous cure all remedy of antiquity<sup>[7,8]</sup>.

However, it remains unknown how Aretaeus made such a precise description of a relatively rare disease during that period, just by observation.

## THOMAS WILLIS (1621-1675) AND THE TERM "MELLITUS"

The English anatomist and physician Thomas Willis, is considered one of the greatest physicians in 17<sup>th</sup> century. He lived in a period that England was in political and religious turmoil and he needed to interrupt several times his studies. Willis studied classics and then medicine at Oxford where he was appointed Professor of Natural Philosophy to the highly prestigious Sedleian chair. During his career, he wrote several books and articles on medicine and his work on the anatomy of the brain and nervous system, based on his own dissections, remains very celebrated. Willis provided the description of the autonomic nervous system, the spinal cord, the vasculature at the base of the brain (circle of Willis) and the cranial nerves, including the accessory nerve (Willis' nerve)<sup>[9]</sup>.

Willis, as physician, belonged to the Iatrochemical School of medicine which believed that chemistry was the basis of human function. Concerning diabetes, in his *Pharmaceutice rationalis*, Willis devoted a chapter to the "pissing evil". He commented on the sweetness of the urine in diabetic patients, coining also the term mellitus<sup>[10]</sup>. It was actually a rediscovery, as in the 7<sup>th</sup> century BC the Indian physician Sushruta mentioned the sweet urine of the disease but this work apparently was unknown to Willis. So, he was the first European medical writer who mentioned the sweet taste of the urine in diabetes mellitus. It seems that he saw several cases of diabetes mellitus and he believed that it was due to an affection of the blood rather of the kidneys. He attributed it to the eating habits and psychological status "an ill manner of living and chiefly an assiduous and immoderate drinking of cider, beer and sharp wines; sometimes sadness, long grief". He recognized also diabetic neuropathy in the sufferers describing it as "stinging and other...frequent contractions or convulsion, twinging of the tendons and muscles and other disturbances"<sup>[9-11]</sup>.

Concerning the sweet taste of the urine, he reported a case of "a certain noble earl" who suddenly "became much inclined to excessive pissing... in the space of twenty-four hours, he voided almost a gallon and a half of limpid, clear, and wonderful sweet water, that tasted as if it has been mixed with honey". Therapeutically he considered beneficial for the disease a "thickening and moderately cooling diet and cordials" and he mentioned that slimy vegetables, rice, white starch may improve patient's status. He also suggested a milk drink which was distilled with cypress tops and egg whites, two



**Figure 2** Portrait of the French physiologist Claude Bernard. (Source: Wellcome Library, London).

powders (a mixture of gum arabic and gum dragant), rhubarb and cinnamon. Following Willis' therapeutic advices, patient's condition improved in a month but immediately after his recovery, he returned to his past dietary habits<sup>[9]</sup>.

However Willis could not explain "why the urine is wonderfully sweet like sugar or honey". The explanation was given 100 years later, by another English physician, Matthew Dobson (1732-1784) of Liverpool, who experimentally demonstrated the presence of sugar in urine. He actually boiled urine to dryness and noticed that the residue, a crystalline material, had the taste of brown sugar<sup>[11]</sup>.

## CLAUDE BERNARD'S (1813-1878) BRILLIANT DISCOVERY ON THE GLYCOGENIC ACTION OF THE LIVER

Born to a poor family in Beaujolais region, south of France, Claude Bernard at the age of 19 was apprenticed to an apothecary. His passion for the theatre led him to write two plays *La Rose du Rhône* and *Arthur de Bretagne* but soon after arriving to Paris, he was discouraged by the literary critic and politician Saint-Marc Girardin (1801-1873) who counseled him to enroll in medicine. In Medical School of Paris, Bernard was not considered a brilliant student and unwilling to practice medicine, he was appointed assistant to the Professor of Physiology and pioneer of experimental physiology François Magendie (1783-1855). However, Bernard's research career was very successful. In 1854, he became member of the Academy of Sciences and later on he succeeded Magendie to the chair of experimental physiology at the College de France. The Emperor Napoleon III admired him so much that created two laboratories for him and made him a Senator. Among

Bernard's several discoveries we may cite: the vasomotor innervation, the principle of physiological determinism, the concept of internal secretion, the concept of milieu intérieur or internal environment (meaning the interstitial fluid, and its physiological capacity to ensure protective stability for the tissues and organs), the nature and function of curare, carbon monoxide and other poisons (Figure 2). Unfortunately, the only way to understand and discover all these phenomena, promoting our knowledge to physiology, was through animals' vivisections. This was the reason for his wife to divorce him and join with his children the antivivisection movement, campaigning actively on the issue<sup>[12]</sup>.

Bernard's contribution in the study of metabolism and diabetes remains leading. In 19<sup>th</sup> century, scientists hypothesized on the role of pancreas in the physio-pathology of diabetes as they found in the post-mortem examination of the diseased, atrophic or stone filled pancreases. However, as they believed that pancreas was an exocrine organ, they interpreted these post-mortem findings as a chance phenomenon. During that period the French experimental physiologist, Claude Bernard decided to test this hypothesis<sup>[1,12]</sup>.

At the beginning, he falsely believed that "diabetes was a nervous affection of the lungs". However, during an experiment, he injected grape sugar into the jugular vein of a dog, extracting at the same time blood from the carotid artery. This blood contained a large amount of sugar and he realized that glucose was not destroyed in the lungs, because blood must pass by these organs in order to move from the jugular vein to the carotid artery. He was then fed dogs on a carbohydrate-rich diet, the blood from the hepatic veins and vena cava contained sugar which was not destroyed in the liver and was also present in heart ventricles, so the theory of lungs' role in diabetes was rejected. In further experiments, Bernard proved that animal blood contains sugar even if it is not supplied by food. Testing the theory that sugar absorbed from food was destroyed when it was passing through tissues, Bernard put dogs in carbohydrate diet and killed them immediately after feeding. To his surprise he observed large amounts of sugar in hepatic veins. The same observation was done in the control group, animals that were fed only by meat. He then moved to the analysis of liver tissue samples and in every liver he examined he found large quantities of glucose which was missing from other organs. He concluded that liver was storing a water insoluble starchy substance that he named glycogen which was converted into sugar or glucose and secreted into the blood. He assumed that it was an excess of this secretion that caused diabetes<sup>[13,14]</sup>.

Moving toward, Bernard demonstrated the connection between the central nervous system and diabetes. Using a needle, he stimulated the floor of the fourth brain ventricle and produced temporary "artificial diabetes" which lasted less than one day. He named this procedure *piqûre diabétique* and linked for the first time glucose homeostasis and the brain to the pathogenesis



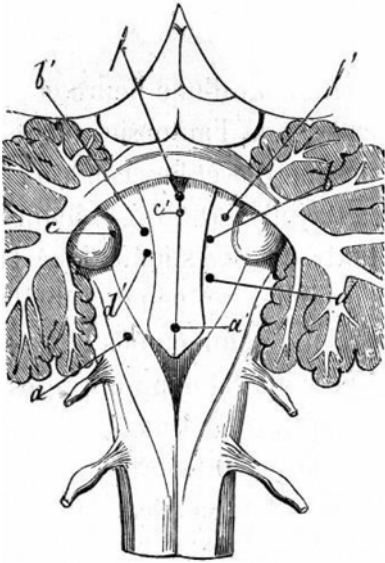


Figure 3 Sites of punctures of 4<sup>th</sup> ventricle from Bernard's book «Leçons sur la Physiologie et la Pathologie du Système Nerveux», 1858. (Source: Wellcome Library, London).

of diabetes<sup>[15]</sup> (Figure 3).

The work of Claude Bernard on glycogenic action of the liver illuminated the pathway of gluconeogenesis and promoted the study of diabetes.

### OSKAR MINKOWSKI (1858-1931) AND JOSEPH VON MERING (1849-1908): THE DISCOVERY OF "PANCREATIC DIABETES"

A turning point in the history of diabetes mellitus took place in 1889 after the experiments of Minkowski and von Mering.

In 1886, three years before their first meeting, von Mering discovered that phlorizin, a glucoside, could cause transient glucuresis. In 1889, while von Mering was working in Hoppe Seyler's Institute at the University of Strasbourg, Minkowski, assistant at that time to the German leading authority on diabetes Professor Bernard Naunyn (1839-1925), he visited the Institute to look at some chemical books of the library. They met accidentally and talked about Lipanin, an oil containing free fatty acids and von Mering used to administrate to patients suffering from digestive disturbances. Minkowski was not in favor of Lipanin intake and then their conversation turned on whether the pancreas had a role in digestion and absorption of fats. As a result of the discussion, the two men decided the same evening to perform a pancreatectomy in a dog in Naunyn's laboratory. The animal remained alive and was closely observed by Minkowski, as von Mering left urgently to Colmar because of a family issue. Soon after the operation, the dog developed polyuria. Minkowski examined the urine and found that it contained 12% sugar. Initially Minkowski believed that



Figure 4 The Nobel laureate Frederick Banting in his laboratory with a dog. (Source: Wellcome Library, London).

the dog developed diabetes due to the fact that von Mering had treated it for a long time with phlorizin. So he repeated the pancreatectomy in three more dogs which had no sugar in their urine previous to operation and all of them developed glycosuria<sup>[13,16]</sup>.

Furthermore Minkowski implanted a small portion of pancreas subcutaneously, in depancreatized dogs, and observed that hyperglycemia was prevented until the implant was removed or had spontaneously degenerated<sup>[13]</sup>.

Minkowski and von Mering experiment demonstrated that pancreas was a gland of internal secretion important for the maintenance of glucose homeostasis. They also paved the way for Banting and Best to conduct their experiments and to meet with success.

### FREDERICK BANTING (1891-1941), CHARLES BEST (1899-1978), JAMES BERTRAM COLLIP (1892-1965) AND JOHN MACLEOD (1876-1935): THE DISCOVERY OF INSULIN

In 1923 the Nobel Prize in Medicine was awarded to Frederick Banting and John MacLeod for the discovery of insulin. It was actually a story of success that provoked a great scientific conflict.

Frederick Banting was a young Canadian surgeon, who was admitted into the laboratory of the eminent biochemist, interested in diabetes, Professor John Macleod, at the University of Toronto<sup>[13]</sup>. In 1920, Moses Barron, physician in Minnesota, published an article on "The relation of the islets of Langerhans to diabetes, with special reference to cases of pancreatic lithiasis<sup>[17]</sup>" which was mentioning that the continuation of experiments of Minkowski and von Mering could lead to

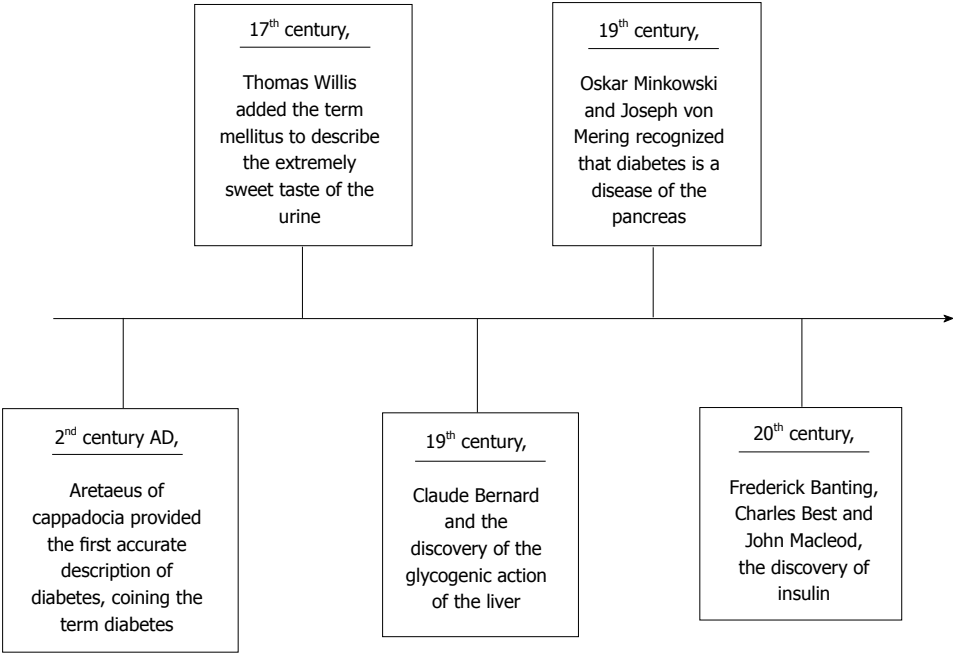


Figure 5 Timeline table presenting the main contributors in the history of diabetes mellitus.

the discovery of a substance capable to control diabetes. Influenced by this article, Banting focused on the study of diabetes<sup>[13]</sup>. During that period the distinguished English physiologist Ernest Starling (1886-1927) was mentioning: “We don’t know yet how the pancreas affects sugar production or utilization in the same animal. It is generally assumed that it secretes into the bloodstream a hormone which may pass to the tissues and enable them to utilize sugar or pass to the liver and inhibit the sugar production of this organ... but we have been unable to imitate the action of the pancreas still in vascular connection with the body, by injection or administration of the extracts of this organ”<sup>[18]</sup>.

On 16 May 1921, Banting started to collaborate with Charles Best, a young medical student. Experimenting in dogs they initially ligate the pancreatic ducts, achieving atrophy of the exocrine region and almost ten weeks later they removed dog’s degenerated pancreas. They crushed the atrophied pancreatic glands in a cool mortar and froze it in salt water. Then the mass was ground down and added to 100 mL of physiological salt. Afterwards, they administrated 5 mL of this extract intravenously to a depangreatized dog. Within 2 h its blood sugar had considerably dropped. They repeated several times the experiment with other diabetic dogs, gaining similar results and they experimented also with fetal calf pancreas using different ways of administration such as subcutaneous and rectal<sup>[19,20]</sup> (Figure 4).

At the end of 1921 the skilled chemist James Collip joined the team and developed a better extraction and purification technique. Obtained substance was initially named by the team insletin and later on by MacLeod insulin<sup>[13]</sup>.

The next step was to test insulin in humans. So on 11 January 1922, insulin was administrated to

Leonard Thompson a 14-year-old boy treated for diabetes in Toronto Hospital<sup>[13]</sup>. It’s worth mentioning that after the introduction of Apollinaire Bouchardat’s (1806-1886) pioneering dietary treatment for diabetes, physicians repeated in several generations of diabetics his motto: “mangez le moins possible” (eat as little as possible)<sup>[21,22]</sup>. Thomson was also following a strict fasting diet proposed by Frederick Madison Allen (1879-1964) and he was in critical state. He received 15 mL of insulin, injected in his buttock but he developed abscesses at the injection site and became even sicker. Collip further improved the quality of insulin and on January 23, Thompson received a second injection. The results were excellent. His blood glucose from 520 mg/dL fell to 120 mg/dL in 24 h and urinary ketones disappeared. Thompson continued the treatment with insulin and lived another 13 years. He died of pneumonia at 27 years old<sup>[13]</sup>. Similar is the story of Elizabeth Hughes Gossett (1907-1981). Daughter of the United States politician Charles Evans Hughes, Elisabeth was diagnosed with diabetes at age 11. Initially she was also treated by Allen and in August 1922 began the use of insulin. She survived, graduated from College, got married, had three children and died suddenly of a heart attack at 74 years old<sup>[23]</sup>.

The pioneering work of Banting and Best saved millions of lives and diabetics started to live a normal life. Lilly Pharmaceutical Company collaborated with the two scientists and in 1923 introduced Iletin, the world’s first commercially available insulin product<sup>[13]</sup>.

However in 1923 the Nobel Committee decided to award Banting and MacLeod for insulin’s discovery. Banting became furious as he believed that he should share the prize with Best instead of MacLeod and he decided to share with Best his cash award. In his turn,

MacLeod shared also his award with Collip<sup>[13]</sup>.

Another black spot in the history of insulin discovery was also the discovery of pancreatin, an extract of bovine pancreas discovered by the Romanian Professor of Physiology Nicolae Constantin Paulescu (1869-1931) in 1916, published a few years later because of the war in 1921 and patented in April 1922. Even if Paulescu was the first to provide a detailed demonstration of the antidiabetic and antiketogenic effect of a pancreatic extract, pancreatine was not used in humans and passed over silently<sup>[24]</sup>.

A crucial step in the history of diabetes has been completed. Over the next years insulin purification methods improved and new insulin formulations were developed such as Protamine-zinc insulin, a long-acting insulin in 1930s, neutral protamine Hagedorn in 1940s and Lente series in 1950s<sup>[13]</sup>.

## CONCLUSION

For more than 3000 years physicians questioned the causes and treatment of diabetes mellitus (Figure 5). However, an important progress has been made over the last two centuries thanks to the development of chemistry, physics and pharmacology. Over the next years scientists continued to make significant discoveries: The structure of insulin was delineated in 1955 by the Nobel laureate Fred Sanger (1918-2013); in 1967 proinsulin was discovered by Donald Steiner (1930-2014) and with his colleagues he produced the radioimmunoassay for C-peptide which is used today to measure endogenous insulin production; in the same year, the first pancreas transplant in a human was performed by William Kelly, Richard Lillehei (1927-1981) and colleagues at the University of Minnesota; in 1972 the U100 insulin was introduced to promote better accuracy in administration; ten years later, in 1982, recombinant human insulin became available and in early 1990's insulin pen delivery devices became popular following by the discoveries of short (1996) and long (2001) acting insulin analogues<sup>[1]</sup>.

Since biotechnology helps medicine to progress, nobody knows what the future will bring. We are sure of just one thing: History of diabetes is being still written.

## REFERENCES

- 1 **Kahn CR**, Weir GC, editors. *Joslin's diabetes mellitus*, 14th ed. Philadelphia: Lippincott, 2005
- 2 Global status report on non communicable diseases 2014. Geneva: World Health Organization, 2012
- 3 **World Health Organization**. Global health estimates: Deaths by cause, age, sex and country, 2000-2012. Geneva: World Health Organization, 2014
- 4 **Mathers CD**, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med* 2006; **3**: e442 [PMID: 17132052 DOI: 10.1371/journal.pmed.0030442]
- 5 **Ghalioungui P**. The Ebers papyrus: A new English translation, commentaries and glossaries. Cairo: Academy of Scientific Research and Technology, 1987
- 6 **Peumery JJ**. Histoire illustrée du diabète. De l'Antiquité à nos jours. Paris: Les Éditions Roger Dacosta, 1987
- 7 **Laios K**, Karamanou M, Saridakis Z, Androutsos G. Aretaeus of Cappadocia and the first description of diabetes. *Hormones (Athens)* 2012; **11**: 109-113 [PMID: 22450352]
- 8 **Adams F**. The extant works of Aretaeus the Cappadocian. London: Sydenham Society, 1856
- 9 **Furdell EL**. Fatal Thirst. Diabetes in Britain until Insulin. Leiden: Brill, 2009: 81-92
- 10 **Willis T**. Opera Omnia. Coloniae: Sumptibus Gasparis Storti, 1694: 460
- 11 **Williamson RT**. English Physicians of the past. Newcastle: Andrew Reid and Company, 1923: 47-52
- 12 **Grmek M**. Le Legs de Claude Bernard. Paris: Fayard, 1997
- 13 **von Engelhardt D**, editor. Diabetes: Its medical and cultural history. Berlin: Springer-Verlag, 1989: 306-319, 350-358, 411-426
- 14 **Bernard C**. Du suc pancréatique et de son rôle dans les phénomènes de la digestion. *C R Soc Acad Sci (Paris)* 1850; **1**: 99-119
- 15 **Grmek M**. Examen critique de la genèse d'une grande découverte: La piqûre diabétique de Claude Bernard. *Clio med* 1965; **1**: 341-350
- 16 **von Mering J**, Minkowski O. Diabetes mellitus nach Pankreas extirpation. *Arch exper Path u Pharmacol* 1889; **26**: 371 [DOI: 10.1007/BF01831214]
- 17 **Barron M**. The relation of the Islets of Langerhans to Diabetes. *Surg Gynecol Obstet* 1920; **31**: 437-448
- 18 **Medvei VC**. The History of Clinical Endocrinology: A Comprehensive Account of Endocrinology from Earliest Times to the Present Day. New York: Parthenon, 1993: 253
- 19 **Banting FG**, Best CH, Collip JB, Campbell WR, Fletcher AA, Macleod JJR, Noble EC. The Effect Produced on Diabetes by Extractions of Pancreas. *Transact Ass Amer Physicians* 1922; **37**: 337
- 20 **Banting FG**, Best CH, Macleod JJR. The internal secretion of the pancreas. *Am J Physiol* 1922; **59**: 479
- 21 **Karamanou M**, Koutsilieris M, Laios K, Marineli F, Androutsos G. Apollinaire Bouchardat (1806-1886): founder of modern Diabetology. *Hormones (Athens)* 2014; **13**: 296-300 [PMID: 24776631]
- 22 **Bouchardat A**. De la glycosurie ou diabète sucré. Son traitement hygiénique. Paris: Baillière, 1875
- 23 **Cooper T**, Ainsberg A. Breakthrough: Elizabeth Hughes, the Discovery of Insulin, and the Making of a Medical Miracle. New York: St Martin's Press, 2010
- 24 **Angelescu C**, Nicolae C. Paulescu: Omul și opera sa medicală. Bucuresti: Vremea, 2009

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## Tipping the balance: Haemoglobinopathies and the risk of diabetes

Henry J Baldwin, Aislinn E Green, Kayleigh M Spellar, Philip J Arthur, Hannah G Phillips, Jeetesh V Patel

Henry J Baldwin, Aislinn E Green, Kayleigh M Spellar, Philip J Arthur, Hannah G Phillips, Jeetesh V Patel, University of Nottingham Medical School, Nottingham NG7 2UH, United Kingdom

Jeetesh V Patel, University of Birmingham Centre for Cardiovascular Sciences, Sandwell and West Birmingham Hospitals NHS Trust, West Midlands B18 7QH, United Kingdom

Jeetesh V Patel, Sandwell Medical Research Unit, Lyndon, Sandwell General Hospital, West Midlands B71 4HJ, United Kingdom

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**Correspondence to:** Dr. Jeetesh V Patel, Sandwell Medical Research Unit, Lyndon, Sandwell General Hospital, West Bromwich, West Midlands B71 4HJ, United Kingdom. [jeeteshp@gmail.com](mailto:jeeteshp@gmail.com)  
 Telephone: +44-121-5073971  
 Fax: +44-121-5073216

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### Abstract

**AIM:** To establish a link between the risk of diabetes with haemoglobinopathies by examining available evidence of the effects of iron and blood glucose homeostasis from molecular to epidemiological perspectives.

**METHODS:** A systematic literature search was performed using electronic literature databases using various search terms. The International Diabetes Federation World Atlas was used to generate a list of populations with high rates of diabetes. PubMed, Scopus and Google Scholar were used to identify which of these populations also had a reported prevalence of haemoglobin abnormalities.

**RESULTS:** Abnormalities in iron homeostasis leads to increases in reactive oxygen species in the blood. This promotes oxidative stress which contributes to peripheral resistance to insulin in two ways: (1) reduced insulin/insulin receptor interaction; and (2)  $\beta$ -cell dysfunction. Hepcidin is crucial in terms of maintaining appropriate amounts of iron in the body and is in turn affected by haemoglobinopathies. Hepcidin also has other metabolic effects in places such as the liver but so far the extent of these is not well understood. It does however directly control the levels of serum ferritin. High serum ferritin is found in obese patients and those with diabetes and a meta-analysis of the various studies shows that high serum ferritin does indeed increase diabetes risk.

**CONCLUSION:** From an epidemiological standpoint, it is plausible that the well-documented protective



effects of haemoglobinopathies with regard to malaria may have also offered other evolutionary advantages. By contributing to peripheral insulin resistance, haemoglobinopathies may have helped to sculpt the so-called “thrifty genotype”, which hypothetically is advantageous in times of famine. The prevalence data however is not extensive enough to provide concrete associations between diabetes and haemoglobinopathies - more precise studies are required.

**Key words:** Diabetes; Ferritin; Haemoglobinopathy; Iron metabolism; Malaria

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**Core tip:** Are diabetes and haemoglobinopathies linked? There is strong evidence to suggest that the processes involved in both iron and blood glucose homeostasis interact with one another. Metabolic disorders involving iron appear to contribute to the pathological process of diabetes at least on a cellular level. This article also examines prevalence data of diabetes and various haemoglobinopathies in certain populations to establish whether there is an association from an epidemiological perspective.

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## INTRODUCTION

There are a number of postulated theories that suggest that there were once evolutionary benefits of certain gene variants that are known to cause disease in modern populations. One such association is demonstrated by the protective nature of Sickle-cell trait in terms of the interruption of the life cycle of *Plasmodia*, which lessens the impact of malaria infection on an individual with such a phenotype<sup>[1]</sup>. It does not seem unreasonable to suggest that there may be other associations that are yet to be discovered. The evolutionary advantages of having higher blood glucose concentrations have been suggested by the “thrifty genotype” hypothesis, *i.e.*, peripheral insulin resistance acting to ration energy in times of famine<sup>[2]</sup>. This article focuses on the associations between iron metabolism and type 2 diabetes mellitus by examining the available evidence. The pathological link between haemoglobin abnormalities and diabetes is investigated in addition to the molecular mechanisms that may be involved. The prevalence of type 2 diabetes has risen in populations who live in regions with antecedently high rates of malaria infection and in ethnic groups who have emigrated from these areas<sup>[3,4]</sup>. Prevalence data of

haemoglobinopathies, iron transport abnormalities and diabetes are examined in order to establish whether populations with high rates of diabetes are more likely to have haemoglobin abnormalities.

## MATERIALS AND METHODS

A systematic literature search was performed using electronic literature databases, PubMed, Web of Knowledge and Cochrane Library. The search terms used included: “diabetes”, “diabetes mellitus”, “diabetes mellitus type 2”, “iron”, “free radicals”, “glucose tolerance”, “insulin resistance”, “insulin”, “resistance”, “sensitivity”, “hepcidin”, “ferritin”. Relevant references from selected articles were also reviewed. The International Diabetes Federation World Atlas was used to generate a list of populations with high rates of diabetes. PubMed, Scopus and Google Scholar were used to identify which of these populations also had a reported prevalence of haemoglobin abnormalities.

## RESULTS

### *Putative link between haemoglobin metabolism and diabetes*

**Oxidative stress, iron and diabetes:** The production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) due to iron overload in humans has been attributed to the Fenton Reaction. This occurs due to the ability of iron to convert between its two oxidative states,  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$ <sup>[5]</sup>. Alternative proposed mechanisms include the Haber-Weiss reaction, with haem iron acting as a catalyst and  $\text{Fe}^{2+}$  as a reactant<sup>[6]</sup>. Antioxidants and detoxifying enzymes are required to maintain careful control of ROS and RNS production. Iron overload can tilt this balance, leading to oxidative stress<sup>[6]</sup>. Oxidants have in turn been shown to cause the release of catalytic iron resulting in the formation of yet more ROS and RNS forming a vicious cycle<sup>[7]</sup>. Oxidative stress is one mechanism speculated to be linked to insulin resistance and abnormal glucose tolerance, as a novel explanation of the link between diabetes and iron overload.

Pancreatic  $\beta$ -cells in fact show particular sensitivity to oxidative stress due to their low expression of antioxidants such as catalase and SOD2<sup>[8]</sup>. The resulting  $\beta$ -cell dysfunction as a consequence of this stress causes decreased expression of transcription factors required for cell maintenance and insulin production<sup>[8]</sup>. Further research has demonstrated that circulating insulin is also directly affected by ROS, affecting the ability of insulin to bind to the insulin receptor<sup>[9]</sup>. The combination of these factors consequently leads to hyperglycaemia and ultimately, the development of diabetes.

Homeostatic mechanisms for preventing damage from iron overload include both the regulation of cytosolic iron by binding to iron regulatory proteins (IRP) and production of the peptide hormone Hepcidin<sup>[8]</sup>.

**Table 1** Comparison of studies examining Hepcidin, Prohepcidin and Serum Ferritin concentrations in individuals with type II diabetes

Ref.	No. of patients with type II diabetes	No. of total participants	Hepcidin concentration	Prohepcidin concentration	Serum Ferritin concentration
Aso <i>et al</i> <sup>[16]</sup> , 2010	104	169	-	Significantly lower than control	Significantly higher than control
Jiang <i>et al</i> <sup>[24]</sup> , 2011	34	64	Significantly higher than control	-	Significantly higher than control
Guo <i>et al</i> <sup>[25]</sup> , 2013	555	1259	No significant difference from control	-	Significantly higher than control
Sam <i>et al</i> <sup>[26]</sup> , 2013	33	66	Significantly lower hepcidin than control	-	Not significantly higher than control

Binding of IRP results in a decrease in iron uptake into the body and an increase in the translation of ferritin, a molecule that sequesters iron within cells<sup>[8]</sup>. High serum ferritin levels are associated with obesity, metabolic syndrome and cardiovascular risk and more recent studies have demonstrated it to be directly associated with diabetes<sup>[10-12]</sup>. Care must be taken however in attributing causality to this relationship. Diabetes is known to be a chronic inflammatory state, and this finding may simply be explained by the fact that ferritin is an acute phase reactant and therefore simply produced as a result of inflammation<sup>[13]</sup>.

Hepcidin has been shown to inhibit cellular iron efflux by binding to ferroportin, an important iron exporter, causing the internalisation and degradation of iron<sup>[14,15]</sup>. Subsequently, hepcidin decreases intestinal iron absorption and prevents the release of iron from macrophages<sup>[16]</sup>. The hepcidin-ferroportin axis is essential to maintaining iron homeostasis, however is still not completely understood<sup>[17]</sup>. Hepcidin is modulated by its inversely proportional relationship to both serum and tissue iron, with iron concentrations being inversely proportional to hepcidin concentration. This balance is essential to maintain iron as demonstrated clinically in patients with hereditary hemochromatosis who have low hepcidin levels and hence have toxic accumulation of iron<sup>[18]</sup>.

The synthesis of hepcidin is mainly within hepatocytes, but has also been noted in pancreatic  $\beta$  cells and the adipose tissue of obese patients<sup>[19,20]</sup>. This may suggest that pancreatic  $\beta$  cells also have a role in iron metabolism in addition to the regulation of glucose and insulin<sup>[19]</sup>. Whilst several studies have investigated levels of circulating hepcidin or prohepcidin (a precursor of hepcidin) in patients with diabetes (Table 1), there is currently no consensus or large scale-studies available, and data relating to the role of hepcidin in this context is limited. Cell culture studies have revealed that glucose induces secretion of hepcidin in INS-1E cultures (a pancreatic  $\beta$  cell model) yet has no effect on HepG2 cell cultures (a hepatocyte model)<sup>[21]</sup>. In contrast, insulin up-regulates hepcidin secretion in HepG2 cell cultures. There was no data found for the effect of insulin on hepcidin secretion by pancreatic  $\beta$  cells<sup>[22]</sup>. A single murine study looked at hepcidin activity during starvation. It proposed that the increased hepcidin secretion seen in such states has a role in preserving

tissue iron and supporting gluconeogenesis in the liver<sup>[23]</sup>. As gluconeogenesis is abnormally induced in obese individuals and those with diabetes, a link between diabetes and hepcidin is possible<sup>[23]</sup>. Whilst it seems likely that hepcidin has a role in the glucose-insulin axis, no firm conclusions are possible with the data currently available. Further exploration of the role of hepcidin could explain whether an elevated serum ferritin is the likely cause or effect of the chronic inflammation seen in diabetes.

The link between abnormal iron metabolism and diabetes is established in those with Sickle-cell disease and haemochromatosis<sup>[27,28]</sup>. However, the effect of iron intake on the risk of healthy individuals developing diabetes and its subsequent clinical progression is much less clear.

Haemochromatosis is known to result from the dysregulation of the body's finely balanced iron metabolism<sup>[29]</sup>. The resulting free iron is known to be toxic when present in sufficiently high concentrations although the exact mechanisms behind its role in both health and disease are still not fully understood. The ubiquitous nature of iron *in vivo*, from oxygen transport and energy metabolism to DNA synthesis, explains the systemic and wide ranging tissue types affected by this disease. Traditional explanations of the resulting diabetes have cited iron as a purely diabetogenic influence<sup>[30]</sup>. However, a recent paper by Abbas *et al*<sup>[31]</sup> challenges the traditional thinking regarding the role that increased iron deposition plays in haemochromatosis. Indeed, iron overload in hereditary haemochromatosis was found to exhibit both pro-diabetic influences, mediated *via* beta-cell toxicity as well as an anti-diabetic effect caused predominately by weight loss<sup>[31]</sup>.

Research targeted at a link between abnormal iron metabolism and diabetes in those who are otherwise healthy has repeatedly produced conflicting results. Jiang *et al*<sup>[32]</sup> conducted a prospective study that followed up a cohort of initially healthy males for 12 years. Total haem and/non-haem iron intake was compared between those who developed diabetes in this time period, and those who remained healthy. Only haem iron was positively associated with diabetes although other lifestyle factors could not be excluded as contributors<sup>[33]</sup>. This result has been backed up by similar research, including data from the Nurses' Health

Study II and other large cohort studies<sup>[34,35]</sup>.

In contrast, an African study demonstrated that there was no link between serum ferritin and diabetes prognosis in those with patients without additional health complications<sup>[36]</sup>. However, with a small sample size ( $n = 60$ ), and the fact that these were not newly diagnosed diabetics this conclusion must also be treated with caution. A study in India concluded there was no link between raised serum ferritin and the risk of developing diabetes. However, it did not look at any other indices of iron status, which would have allowed comparison with the current literature<sup>[37]</sup>.

Orban *et al.*<sup>[33]</sup> recently attempted to make sense of these conflicting results with a meta-analysis of studies of indices of iron status in those without haemochromatosis or thalassemia<sup>[32]</sup>. It concluded that a significant link between a raised ferritin level and an increased risk of diabetes does indeed exist. Other indices such as transferrin saturation and soluble transferrin receptor number were also implicated but a methodology which failed to address the confounding effect of inflammation and a low statistical power means these conclusions must be met with caution<sup>[32]</sup>.

It has been highlighted that these results indicate the very immediate need for further, high quality research regarding the effect of iron intake on the progression of diabetes in those without abnormal iron metabolism<sup>[38]</sup>. For example, looking at the effect of iron supplementation on diabetes progression in newly diagnosed patients. To date only the risk of developing the disease has been looked at in detail epidemiologically. Additionally, the mechanistic studies are generally in their infancy, *i.e.*, are only based on animal models at this stage. This area of research would need to be advanced to human based studies to yield more significant data.

### **Epidemiology of Fe transport/haemoglobin abnormalities and association with diabetes in populations**

The worldwide distribution of the common haemoglobinopathies coincides with that of malaria, and indeed confers resistance from its more severe expressions<sup>[1,39]</sup>. Inherited haemoglobin disorders (Sickle-cell disorders and thalassaemias) were originally characteristic of the tropics and subtropics but are now common worldwide due to migration<sup>[40]</sup>. However, the main regions with the highest rates of Sickle-cell disease are sub-Saharan Africa, the Mediterranean<sup>[41]</sup>, the Middle East<sup>[42,43]</sup> and the Indian subcontinent. Additionally, the Sickle-cell gene variants are extremely common in some of the Caribbean Islands and in North America<sup>[44]</sup>.

The prevalence of diabetes in sub-Saharan Africa is reported as being between 1% (rural Uganda) and 12% (Nairobi)<sup>[45]</sup>. A paediatric study of 860 individuals in Western Kenya reported 38.5% were heterozygous and 9.5% homozygous for  $\alpha$ -thalassaemia. Sickle-cell trait was present in 17.2% and Sickle-cell disease in 1.8%<sup>[46]</sup>. This demonstrates a relatively high prevalence of both diabetes and haemoglobinopathies, calling for

the need for further investigation to directly compare diabetes and haemoglobinopathies in each of these populations. Prevalence of diabetes in India is 9.1%, with the cumulative gene frequency of haemoglobinopathies being 4.2%, with large variation between different ethnic groups<sup>[47]</sup>. Again, direct study of both conditions in these individual ethnic groups is needed in order to draw more meaningful comparison. Turkey is of particular interest as the prevalence of diabetes is 14.8%, but Sickle-cell disease is only found in 0.3%, which suggests much less of a correlation than that seen in India and Africa. However, in some areas of Turkey, (*i.e.*, Çukurova) the prevalence of carriers of HbAS is as high as 44%<sup>[48,49]</sup>. A similar affect has been reported in Madang in Papa New Guinea, where 97% of the population tested were either heterozygous or homozygous for  $\alpha$ -thalassaemia<sup>[50]</sup>. The overall prevalence of diabetes in Papa New Guinea is 5.2%<sup>[51]</sup>, however it would be interesting to examine the populations of Madang and Çukurova for diabetes prevalence specifically due to the extremely high rates of  $\alpha$ -thalassaemia and HbAS. The United States provides interesting data. The overall prevalence of diabetes is 9.2%, with 13.2% of African Americans affected<sup>[52]</sup>. The highest rates of diabetes in the United States are actually amongst American Indians and Alaskan natives (15.9%)<sup>[52]</sup>, where the prevalence of Sickle-cell disease is 36.2/100000 live births, making these ethnic groups the third most affected by Sickle-cell disease behind African Americans (289/100000) and Hispanics (89.1/100000)<sup>[53,54]</sup>. Hb-E occurs widely throughout the eastern half of the Indian subcontinent, Bangladesh, Myanmar, and East and Southeast Asia. Most notably in the Northern parts of Thailand and Cambodia, where the region is referred to as the "Hb-E Triangle" where up to 70% are carriers. The prevalence of diabetes in these areas is 8.5% (Thailand)<sup>[55]</sup> and 2.6% (Cambodia)<sup>[56]</sup>.

Although, on the whole, it is difficult to determine any firm correlations using the above sources, the existing data certainly summons enough intrigue to warrant further investigation.

## **DISCUSSION**

Examining the epidemiological evidence for an association between diabetes and the various haemoglobinopathies is not straightforward. The main issue is the complex interplay of various environmental and biological factors that all contribute to the development of diabetes, making a clear association between certain factors difficult to prove. There is clear evidence on a molecular level of an interaction between glucose homeostasis and haem abnormalities, however the epidemiological perspective remains unclear due to a lack of specific studies in this area. Focussed diabetes prevalence data from the groups with extremely high carrier rates of the various haemoglobinopathies would be extremely beneficial, as a link between the molecular

evidence and the epidemiological picture could be demonstrated. Other issues include the large number of individuals with diabetes who are undiagnosed. Improvements in screening and healthcare education programs seem to be the answer here, although these are not without their own problems. The Center for Disease Control and Prevention estimates this figure to be 8.1 million people (27.8% of those with diabetes) in the United States<sup>[53]</sup>, making true prevalence data difficult to obtain. It remains plausible however that in the face of various selective pressures there was once an evolutionary advantage in having a higher blood glucose level. This could help to explain why there are a number of ethnic groups who are at greater risk of developing diabetes than others. It is also possible that these genetic predispositions to higher blood glucose levels developed in tandem with the haem abnormalities that are known to be protective against malaria. However with a lack of studies directly examining the two conditions, a concrete association is difficult to prove.

## COMMENTS

### Background

A putative pathophysiological mechanism exists between diabetes and blood born disorders. These processes involve both iron and blood glucose metabolism and there is a high potential for the two to interact with one another.

### Research frontiers

Metabolic disorders involving iron contribute to diabetes on a cellular level. Evidence at a clinical or population level is less clear and is reviewed here.

### Innovations and breakthroughs

The evidence reviewed here provides a putative link between diabetes and haemoglobinopathies which carries clinical ramifications (with respect to risk) for populations that have an antecedent risk of blood born disorders. The role of iron metabolism and its impact on diabetogenic risk is also considered here.

### Peer-review

This article is based on a literature search, focusing on correlations between iron metabolism and type 2 diabetes, and on epidemiological data in search for a possible link between diabetes and haemoglobinopathies. It is a potentially useful paper for discussion of an important subject that could be of use to the clinicians and researchers in the field as an overview, where many studies are compared with their strong and weak points, and suggestions are given.

## REFERENCES

- 1 Taylor SM, Parobek CM, Fairhurst RM. Haemoglobinopathies and the clinical epidemiology of malaria: a systematic review and meta-analysis. *Lancet Infect Dis* 2012; **12**: 457-468 [PMID: 22445352 DOI: 10.1016/S1473-3099(12)70055-5]
- 2 NEEL JV. Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"? *Am J Hum Genet* 1962; **14**: 353-362 [PMID: 13937884]
- 3 Mbanya JC, Motala AA, Sobngwi E, Assah FK, Enoru ST. Diabetes in sub-Saharan Africa. *Lancet* 2010; **375**: 2254-2266 [PMID: 20609971 DOI: 10.1016/S0140-6736(10)60550-8]
- 4 Oza-Frank R, Narayan KM. Overweight and diabetes prevalence among US immigrants. *Am J Public Health* 2010; **100**: 661-668 [PMID: 19608956 DOI: 10.2105/ajph.2008.149492]
- 5 Papanikolaou G, Pantopoulos K. Iron metabolism and toxicity. *Toxicol Appl Pharmacol* 2005; **202**: 199-211 [PMID: 15629195 DOI: 10.1016/j.taap.2004.06.021]
- 6 Liu Q, Sun L, Tan Y, Wang G, Lin X, Cai L. Role of iron deficiency and overload in the pathogenesis of diabetes and diabetic complications. *Curr Med Chem* 2009; **16**: 113-129 [PMID: 19149565 DOI: 10.2174/092986709787002862]
- 7 Swaminathan S, Fonseca VA, Alam MG, Shah SV. The role of iron in diabetes and its complications. *Diabetes Care* 2007; **30**: 1926-1933 [PMID: 17429063 DOI: 10.2337/dc06-2625]
- 8 Simcox JA, McClain DA. Iron and diabetes risk. *Cell Metab* 2013; **17**: 329-341 [PMID: 23473030 DOI: 10.1016/j.cmet.2013.02.007]
- 9 Montes-Cortes DH, Hicks JJ, Ceballos-Reyes GM, Garcia-Sanchez JR, Medina-Navarro R, Olivares-Corichi IM. Chemical and functional changes of human insulin by in vitro incubation with blood from diabetic patients in oxidative stress. *Metabolism* 2010; **59**: 935-942 [PMID: 20022071 DOI: 10.1016/j.metabol.2009.10.013]
- 10 Yeap BB, Divitini ML, Gunton JE, Olynyk JK, Beilby JP, McQuillan B, Hung J, Knuiman MW. Higher ferritin levels, but not serum iron or transferrin saturation, are associated with Type 2 diabetes mellitus in adult men and women free of genetic haemochromatosis. *Clin Endocrinol (Oxf)* 2015; **82**: 525-532 [PMID: 24953981 DOI: 10.1111/cen.12529]
- 11 Vari IS, Balkau B, Kettaneh A, André P, Tichet J, Fumeron F, Caces E, Marre M, Grandchamp B, Ducimetière P. Ferritin and transferrin are associated with metabolic syndrome abnormalities and their change over time in a general population: Data from an Epidemiological Study on the Insulin Resistance Syndrome (DESIR). *Diabetes Care* 2007; **30**: 1795-1801 [PMID: 17416791 DOI: 10.2337/dc06-2312]
- 12 Bao W, Rong Y, Rong S, Liu L. Dietary iron intake, body iron stores, and the risk of type 2 diabetes: a systematic review and meta-analysis. *BMC Med* 2012; **10**: 119 [PMID: 23046549 DOI: 10.1186/1741-7015-10-119]
- 13 Zimmermann MB. Methods to assess iron and iodine status. *Br J Nutr* 2008; **99** Suppl 3: S2-S9 [PMID: 18598585 DOI: 10.1017/S000711450800679X]
- 14 Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM, Ganz T, Kaplan J. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science* 2004; **306**: 2090-2093 [PMID: 15514116 DOI: 10.1126/science.1104742]
- 15 Ganz T. Hepcidin and iron regulation, 10 years later. *Blood* 2011; **117**: 4425-4433 [PMID: 21346250 DOI: 10.1182/blood-2011-01-258467]
- 16 Aso Y, Takebayashi K, Wakabayashi S, Momobayashi A, Sugawara N, Terasawa T, Naruse R, Hara K, Suetsugu M, Morita K, Inukai T. Relation between serum high molecular weight adiponectin and serum ferritin or prohepcidin in patients with type 2 diabetes. *Diabetes Res Clin Pract* 2010; **90**: 250-255 [PMID: 20888657 DOI: 10.1016/j.diabres.2010.09.008]
- 17 Camaschella C. Iron and hepcidin: a story of recycling and balance. *Hematology Am Soc Hematol Educ Program* 2013; **2013**: 1-8 [PMID: 24319154 DOI: 10.1182/asheducation-2013.1.1]
- 18 Pietrangolo A. Hereditary hemochromatosis: pathogenesis, diagnosis, and treatment. *Gastroenterology* 2010; **139**: 393-408, 408.e1-2 [PMID: 20542038 DOI: 10.1053/j.gastro.2010.06.013]
- 19 Kulaksiz H, Fein E, Redecker P, Stremmel W, Adler G, Cetin Y. Pancreatic beta-cells express hepcidin, an iron-uptake regulatory peptide. *J Endocrinol* 2008; **197**: 241-249 [PMID: 18434354 DOI: 10.1677/JOE-07-0528]
- 20 Gotardo EM, dos Santos AN, Miyashiro RA, Gambero S, Rocha T, Ribeiro ML, Gambero A. Mice that are fed a high-fat diet display increased hepcidin expression in adipose tissue. *J Nutr Sci Vitaminol (Tokyo)* 2013; **59**: 454-461 [PMID: 24418880 DOI: 10.3177/jnsv.59.454]
- 21 Aigner E, Felder TK, Oberkofler H, Hahne P, Auer S, Soyak S, Stadlmayr A, Schwenoha K, Pirich C, Hengster P, Datz C, Patsch W. Glucose acts as a regulator of serum iron by increasing serum hepcidin concentrations. *J Nutr Biochem* 2013; **24**: 112-117 [PMID: 22819549 DOI: 10.1016/j.jnutbio.2012.02.017]
- 22 Wang H, Li H, Jiang X, Shi W, Shen Z, Li M. Hepcidin is directly regulated by insulin and plays an important role in iron overload in



- streptozotocin-induced diabetic rats. *Diabetes* 2014; **63**: 1506-1518 [PMID: 24379355 DOI: 10.2337/db13-1195]
- 23 **Vecchi C**, Montosi G, Garuti C, Corradini E, Sabelli M, Canali S, Pietrangelo A. Gluconeogenic signals regulate iron homeostasis via hepcidin in mice. *Gastroenterology* 2014; **146**: 1060-1069 [PMID: 24361124 DOI: 10.1053/j.gastro.2013.12.016]
  - 24 **Jiang F**, Sun ZZ, Tang YT, Xu C, Jiao XY. Hepcidin expression and iron parameters change in Type 2 diabetic patients. *Diabetes Res Clin Pract* 2011; **93**: 43-48 [PMID: 21513996 DOI: 10.1016/j.diabres.2011.03.028]
  - 25 **Guo X**, Zhou D, An P, Wu Q, Wang H, Wu A, Mu M, Zhang D, Zhang Z, Wang H, He L, Liu Y, Wang F. Associations between serum hepcidin, ferritin and Hb concentrations and type 2 diabetes risks in a Han Chinese population. *Br J Nutr* 2013; **110**: 2180-2185 [PMID: 23742704 DOI: 10.1017/S0007114513001827]
  - 26 **Sam AH**, Busbridge M, Amin A, Webber L, White D, Franks S, Martin NM, Sleeth M, Ismail NA, Daud NM, Papamargaritis D, Le Roux CW, Chapman RS, Frost G, Bloom SR, Murphy KG. Hepcidin levels in diabetes mellitus and polycystic ovary syndrome. *Diabet Med* 2013; **30**: 1495-1499 [PMID: 23796160 DOI: 10.1111/dme.12262]
  - 27 **Kunutsor SK**, Apekey TA, Walley J, Kain K. Ferritin levels and risk of type 2 diabetes mellitus: an updated systematic review and meta-analysis of prospective evidence. *Diabetes Metab Res Rev* 2013; **29**: 308-318 [PMID: 23381919 DOI: 10.1002/dmrr.2394]
  - 28 **Zhao Z**, Li S, Liu G, Yan F, Ma X, Huang Z, Tian H. Body iron stores and heme-iron intake in relation to risk of type 2 diabetes: a systematic review and meta-analysis. *PLoS One* 2012; **7**: e41641 [PMID: 22848554 DOI: 10.1371/journal.pone.0041641]
  - 29 **Fernández-Real JM**, Manco M. Effects of iron overload on chronic metabolic diseases. *Lancet Diabetes Endocrinol* 2014; **2**: 513-526 [PMID: 24731656 DOI: 10.1016/S2213-8587(13)70174-8]
  - 30 **Gochee PA**, Powell LW. What's new in hemochromatosis. *Curr Opin Hematol* 2001; **8**: 98-104 [PMID: 11224684 DOI: 10.1097/00062752-200103000-00007]
  - 31 **Abbas MA**, Abraham D, Kushner JP, McClain DA. Anti-obesity and pro-diabetic effects of hemochromatosis. *Obesity* (Silver Spring) 2014; **22**: 2120-2122 [PMID: 25044717 DOI: 10.1002/oby.20839]
  - 32 **Jiang R**, Ma J, Ascherio A, Stampfer MJ, Willett WC, Hu FB. Dietary iron intake and blood donations in relation to risk of type 2 diabetes in men: a prospective cohort study. *Am J Clin Nutr* 2004; **79**: 70-75 [PMID: 14684399]
  - 33 **Orban E**, Schwab S, Thorand B, Huth C. Association of iron indices and type 2 diabetes: a meta-analysis of observational studies. *Diabetes Metab Res Rev* 2014; **30**: 372-394 [PMID: 24327370 DOI: 10.1002/dmrr.2506]
  - 34 **Aune D**, Ursin G, Veierød MB. Meat consumption and the risk of type 2 diabetes: a systematic review and meta-analysis of cohort studies. *Diabetologia* 2009; **52**: 2277-2287 [PMID: 19662376 DOI: 10.1007/s00125-009-1481-x]
  - 35 **Pan A**, Sun Q, Bernstein AM, Schulze MB, Manson JE, Willett WC, Hu FB. Red meat consumption and risk of type 2 diabetes: 3 cohorts of US adults and an updated meta-analysis. *Am J Clin Nutr* 2011; **94**: 1088-1096 [PMID: 21831992 DOI: 10.3945/ajcn.111.018978]
  - 36 **Nubila T**, Ukaejiofo EO, Ike SO, Shu EN, Nubila NI, Chijioke CP, Ukaejiofo AC, Iyare EE, Okwosa CU, Okwuowulu OV. Predisposing factors associated with uncomplicated type 2 diabetes among adults in a diabetic clinic, Enugu State, Nigeria. *Trans R Soc Trop Med Hyg* 2014; **108**: 206-212 [PMID: 24627425 DOI: 10.1093/trstmh/tru024]
  - 37 **Gupta M**, Palta A, Singh R, Lehl SS. Body iron stores in middle-aged North Indian patients with type 2 diabetes and obesity. *J Midlife Health* 2014; **5**: 72-77 [PMID: 24970985 DOI: 10.4103/0976-7800.133991]
  - 38 **Shah SV**, Fonseca VA. Iron and diabetes revisited. *Diabetes Care* 2011; **34**: 1676-1677 [PMID: 21709301 DOI: 10.2337/dc11-0700]
  - 39 **Flint J**, Harding RM, Boyce AJ, Clegg JB. The population genetics of the haemoglobinopathies. *Baillieres Clin Haematol* 1998; **11**: 1-51 [PMID: 10872472 DOI: 10.1016/S0950-3536(98)80069-3]
  - 40 **Modell B**, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. *Bull World Health Organ* 2008; **86**: 480-487 [PMID: 18568278 DOI: 10.2471/BLT.06.036673]
  - 41 **Williams TN**, Weatherall DJ. World distribution, population genetics, and health burden of the hemoglobinopathies. *Cold Spring Harb Perspect Med* 2012; **2**: a011692 [PMID: 22951448 DOI: 10.1101/cshperspect.a011692]
  - 42 **Angastiniotis M**, Modell B. Global epidemiology of hemoglobin disorders. *Ann N Y Acad Sci* 1998; **850**: 251-269 [PMID: 9668547 DOI: 10.1111/j.1749-6632.1998.tb10482.x]
  - 43 **Weatherall D**. 2003 William Allan Award address. The Thalassemias: the role of molecular genetics in an evolving global health problem. *Am J Hum Genet* 2004; **74**: 385-392 [PMID: 15053011 DOI: 10.1086/381402]
  - 44 **Weatherall DJ**, Clegg JB. Inherited haemoglobin disorders: an increasing global health problem. *Bull World Health Organ* 2001; **79**: 704-712 [PMID: 11545326]
  - 45 **Hall V**, Thomsen RW, Henriksen O, Lohse N. Diabetes in Sub Saharan Africa 1999-2011: epidemiology and public health implications. A systematic review. *BMC Public Health* 2011; **11**: 564 [PMID: 21756350 DOI: 10.1186/1471-2458-11-564]
  - 46 **Suchdev PS**, Ruth LJ, Earley M, Macharia A, Williams TN. The burden and consequences of inherited blood disorders among young children in western Kenya. *Matern Child Nutr* 2014; **10**: 135-144 [PMID: 22973867 DOI: 10.1111/j.1740-8709.2012.00454.x]
  - 47 **Urade BP**. Incidence of Sick Cell Anaemia and Thalassemia in Central India. *Open J Blood Dis* 2012; **2**: 71-80 [DOI: 10.4236/ojbd.2012.24014]
  - 48 **Altay Ç**. Abnormal hemoglobins in Turkey. *Turk J Hematol* 2002; **19**: 63-74
  - 49 **Çürük MA**, Yalin E, Aksoy K. Prevention of hemoglobinopathies in Turkey. *Thalassemia Reports* 2013; **3**: e1 [DOI: 10.4081/thal.2013.e1]
  - 50 **Yenchitsomanus PT**, Summers KM, Bhatia KK, Cattani J, Board PG. Extremely high frequencies of alpha-globin gene deletion in Madang and on Kar Kar Island, Papua New Guinea. *Am J Hum Genet* 1985; **37**: 778-784 [PMID: 9556666]
  - 51 **International Diabetes Federation Western Pacific**. Diabetes in Papua New Guinea, 2014. [accessed 2015 May]. Available from: URL: <http://www.idf.org/membership/wp/papua-new-guinea>
  - 52 **Centers for Disease Control and Prevention**. National Diabetes Statistics Report, 2014. [accessed 2015 May]. Available from: URL: <http://www.cdc.gov/diabetes/pubs/statsreport14/national-diabetes-report-web.pdf>
  - 53 **Prabhakar H**. Sick Cell Disease and Native Americans: Overview and Long-Term Considerations for Delivery of Care. *The IHS Primary Care Provider* 2009; **34**: 309-313
  - 54 **Centers for Disease Control and Prevention**. Sick Cell Disease, 2011. [accessed 2015 May]. Available from: URL: <http://www.cdc.gov/ncbddd/sicklecell/data.html>
  - 55 **International Diabetes Federation Western Pacific**. Diabetes in Thailand, 2014. [accessed 2015 May]. Available from: URL: <http://www.idf.org/membership/wp/thailand>
  - 56 **International Diabetes Federation Western Pacific**. Diabetes in Cambodia, 2014. [accessed 2015 May]. Available from: URL: <http://www.idf.org/membership/wp/cambodia>

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## Brain changes in diabetes mellitus patients with gastrointestinal symptoms

Anne M Drewes, Eirik Søfteland, Georg Dimcevski, Adam D Farmer, Christina Brock, Jens B Frøkjær, Klaus Krogh, Asbjørn M Drewes

Anne M Drewes, Eirik Søfteland, Georg Dimcevski, Department of Medicine, Haukeland University Hospital, 5021 Bergen, Norway

Anne M Drewes, Adam D Farmer, Centre for Digestive Diseases, Blizard Institute of Cell and Molecular Science, Wingate Institute of Neurogastroenterology, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London E1 2AJ, United Kingdom

Adam D Farmer, Department of Gastroenterology, University Hospitals of North Midlands, Stoke on Trent, Staffordshire ST4 6QG, United Kingdom

Christina Brock, Jens B Frøkjær, Asbjørn M Drewes, Mech-Sense, Department of Gastroenterology and Hepatology and Clinical Institute, Aalborg University Hospital, 9100 Aalborg, Denmark

Jens B Frøkjær, Department of Radiology, Aalborg University Hospital, 9100 Aalborg, Denmark

Klaus Krogh, Neurogastroenterology Unit, Department of Hepatology and Gastroenterology, Aarhus University Hospital, 8000 Aarhus, Denmark

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Correspondence to: Asbjørn M Drewes, PhD, DMSc, Professor, Mech-Sense, Department of Gastroenterology and Hepatology and Clinical Institute, Aalborg University Hospital, Mølleparkvej 4, 9000 Aalborg, Denmark. [amd@rn.dk](mailto:amd@rn.dk)  
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### Abstract

Diabetes mellitus is a common disease and its prevalence is increasing worldwide. In various studies up to 30%-70% of patients present dysfunction and complications related to the gut. To date several clinical studies have demonstrated that autonomic nervous system neuropathy and generalized neuropathy of the central nervous system (CNS) may play a major role. This systematic review provides an overview of the neurodegenerative changes that occur as a consequence of diabetes with a focus on the CNS changes and gastrointestinal (GI) dysfunction. Animal models where diabetes was induced experimentally support that the disease induces changes in CNS. Recent investigations with electroencephalography and functional brain imaging in patients with diabetes confirm these structural and functional brain changes. Encephalographic studies demonstrated that altered insular processing of sensory stimuli seems to be a key player in symptom generation. In fact one study indicated that the more GI symptoms the patients experienced, the deeper the insular electrical source was located. The electroencephalography was often used in combination with quantitative sensory testing

mainly showing hyposensitivity to stimulation of GI organs. Imaging studies on patients with diabetes and GI symptoms mainly showed microstructural changes, especially in brain areas involved in visceral sensory processing. As the electrophysiological and imaging changes were associated with GI and autonomic symptoms they may represent a future therapeutic target for treating diabetics either pharmacologically or with neuromodulation.

**Key words:** Diabetes mellitus; Gastrointestinal; Electroencephalogram; Magnetic resonance imaging; Brain

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**Core tip:** Investigation of the existing literature on diabetes patients with gastrointestinal (GI) symptoms indicates the presence of structural and functional brain changes. This was most consistent in electrophysiological studies, where especially changes in the insula seemed to correlate with GI symptoms. Imaging studies confirmed the electrophysiological findings showing microstructural changes in brain areas involved in visceral sensory processing. Due to these findings, future targets in treatment of GI symptoms in patients with diabetes may be based on modulation of central nervous system reorganisation, either pharmacologically or with afferent nerve stimulation.

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## INTRODUCTION

According to the World Health Organization diabetes mellitus (DM) is a common disease with a global prevalence estimated to be 9% among adults aged 18+ years. In various studies up to 30%-70% of DM patients complain of gastrointestinal (GI) dysfunction and complications<sup>[1-3]</sup>. This can be manifested as for example vomiting, diarrhoea, abdominal discomfort, constipation and faecal incontinence<sup>[4]</sup>. DM related GI dysfunction may also impair glucose control and increase the risk of malnutrition, which again leads to poor quality of life, weight loss and emptying of glucagon deposits<sup>[5]</sup>. Therefore it is of major importance to focus on the GI complications in patients with DM.

The GI symptoms have in several clinical studies been shown to relate to *peripheral* diabetic autonomic neuropathy, including the enteric nervous system<sup>[6]</sup>. However, as the neuropathy is generalized the central nervous system (CNS) may play a role as well. Hence, the cerebral complications of both type 1 and

type 2 diabetes have been referred to as "diabetic encephalopathy", a term introduced several decades ago<sup>[7]</sup>.

The pathophysiology behind generalized neuropathy is multifactorial, with metabolic, oxidative or immune-related damage of the neurons or glia cells as main factors. Apart from effects on the enteric nervous system that may lead to dysmotility, *etc.*, specific symptoms such as vomiting and nausea are mainly controlled from the brain. Therefore dysfunction of the CNS is mandatory to consider, when other reasons for vomiting and nausea have been ruled out. Furthermore, although some GI motility is present even in patients with severe CNS damage, it is partly centrally regulated, and therefore brain changes will invariably have an effect on gut function<sup>[8]</sup>.

What is already known on this area: (1) diabetes mellitus can cause peripheral and autonomous neuropathies; (2) 50% of patients with longstanding diabetes suffer from GI symptoms; and (3) the symptoms lead to severe socio-economic problems and reduced quality of life.

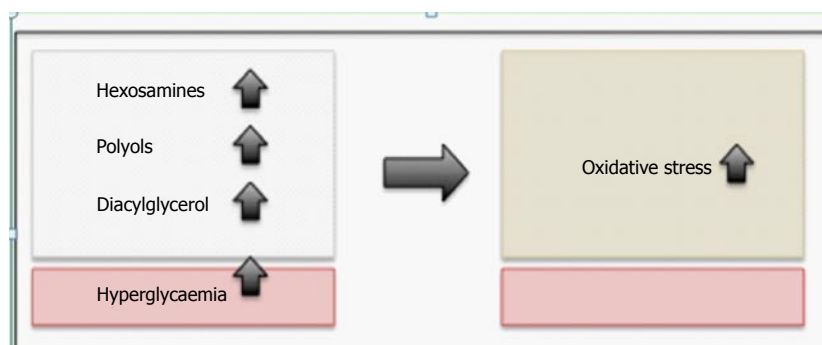
The aim of this review: (1) to update the literature about diabetes and brain changes in humans; and (2) to provide evidence that central neuroplastic and structural alterations may play a major role in diabetic patients with GI symptoms.

Several recent papers focusing on changes in the brain support the above considerations. Hence, in animals with diabetes, changes in the paraventricular nuclei of the hypothalamus as well as the dorsal motor nuclei have been found<sup>[9]</sup>. Changes in these areas are related to the function of the GI tract. In diabetes patients with GI symptoms changes of brain areas, which are involved in visceral sensory processing has been reported. Thus, novel methods used to treat vomiting in DM such as gastric electrical stimulation may exert its effects mainly *via* the brain since no clear effect and the gastric motor function is seen<sup>[10]</sup>. However significant knowledge gaps remain, but addressing central alterations may provide new insight which may guide future therapeutic targets for treatment of GI complications in DM<sup>[11]</sup>.

The aim of this review is to explore the current literature investigating brain changes in patients with DM and GI symptoms. Furthermore, the background for metabolic brain changes, neurophysiological and imaging methods will briefly be discussed.

## LITERATURE SEARCH

PubMed searches were performed for articles and abstracts published in English. There was no lower limit for the time of publication, but literature was searched up to January 2015. Although the focus of this review was studies in humans, animal studies are cited where they illustrate a point of importance. Medical sub-heading (MeSH) and free-text terms for "CNS", "brain", "electroencephalogram" (EEG), and "magnetic



**Figure 1** Hyperglycaemia leads to increased hexosamines, polyols and diacylglycerol within the cell, which can cause oxidative stress leading to cell damage.

resonance imaging (MRI)" were combined with "DM" and "GI symptoms". The authors reviewed titles and abstracts to identify studies examining brain changes in DM with GI symptoms. In addition to the structured literature search a manual search of references from articles included was also conducted. Thus, a number of articles not identified by the original search were included in this review if all other requirements were met. Mainly studies examining neurodegenerative changes in DM patients with GI symptoms were included. The level of evidence was not graded due to the exploratory nature of many of the studies.

## DIABETIC NEUROPATHY: THE PATHOPHYSIOLOGY

A multiple of factors appear to be involved in the pathogenesis of brain changes in DM. A widely held belief is that CNS changes are secondary to the peripheral neuropathy, because the reduced afferent activity may cause adaptive shrinking<sup>[12]</sup>. However, considerable controversy as to the underlying mechanism remain<sup>[13]</sup>, and an alternative hypothesis is that the CNS changes might indeed be a primary phenomenon. There are several potential causes for the direct changes such as fluctuations in insulin and blood sugar levels, as well as cerebrovascular alterations.

### *The hyperglycaemia hypothesis*

Most research focused on hyperglycaemia and hyperlipidaemia as main players to induce oxidative stress and pro-inflammatory mechanisms. Diabetes-related hyperglycaemia as well as hyperlipidaemia induce a number of pathological changes in neuronal tissue leading to oxidative stress and pro-inflammatory mechanisms, as shown in Figure 1<sup>[14,15]</sup>. Hyperglycaemia leads to elevated intracellular glucose and cellular toxicity. This glucotoxicity alters cell function in different ways leading to increased synthesis of polyols, diacylglycerol (which in turn activates protein kinase C) and hexosamines that accumulate intracellularly<sup>[16]</sup>. The exact mechanism by which these factors cause altered cell function is not yet clear, but they act in concert to induce oxidative stress<sup>[5]</sup>. Thus, levels of free radicals, such as superoxide and nitrogen species rise, especially in the mitochondria. Meanwhile, the ability to scavenge free radicals is reduced because of a depletion of the

proton donor nicotinamide-adenine-dinucleotide<sup>[14]</sup>.

These processes may also trigger an enzyme, poly(ADP-ribose) polymerase, of great importance to deoxyribonucleic acid repair, and thereby cause break-up of the deoxyribonucleic acid strands. A further consequence is the reduction of intracellular nicotinamide-adenine-dinucleotide, exacerbated by the polyol pathway induction. As an end result, adenosine triphosphate levels have been shown to reach critical low levels in, e.g., Schwann cells, possibly resulting in cell death<sup>[17]</sup>. Finally, a key enzyme in glycolysis (glyceraldehyde-3-phosphate dehydrogenase) is inhibited when the superoxide levels rise, which cause a reduction in the substrate flux into the mitochondria. However, glucose still enters the cell, which causes even more activity in the alternative metabolic routes, leading to further production of hexosamines, polyols, poly(ADP-ribose) polymerase and advanced glycation end products, thus closing the loop of a vicious cycle<sup>[18]</sup>. For further details see<sup>[17-19]</sup>.

The hyperglycaemia theory seems more valid for type 1 than type 2 DM. In line with this a Cochrane review showed that improved glucose control inhibits onset of neuropathy in type 1 DM, whereas it only had a modest, non-significant relative risk reduction in patients with type 2 DM after 4 years of follow-up. However, when patients were followed for 15-year the effect of increased glucose control showed significant risk reduction<sup>[20-22]</sup>. Although peripheral neuropathy was explored, the mechanisms are likely similar for other nerve tissues. Hence, the prevention of hyperglycemia is likely also of importance to protect the autonomic nervous system and the brain.

The altered function of the cell can also be caused by other factors. The intracellular non-enzymatic glycation of proteins gives rise to advanced glycation end-products, which in the extracellular matrix interacts with various receptors from matrix to endothelial receptors. This last mentioned interaction can lead to proinflammatory gene expression<sup>[19]</sup>.

### *The influence of severe hypoglycaemia*

Severe and prolonged hypoglycaemia can increase release of excitatory amino acids. The release may turn uncontrolled and trigger calcium influx, thereby leading to activation of proteolytic enzymes, causing neuronal damage<sup>[23]</sup>. However, the brain may utilise



other non-glucose resources such as ketone bodies and amino acids and hence be protected against the hypoglycaemic changes<sup>[7,24]</sup>. This is in contrast to the brain damage caused by ischemia and hypoxia. On the other hand, hypoglycaemia and the counter-regulatory hormonal responses are associated with an acute rise in haematocrit levels and blood viscosity, and this may influence capillary blood flow especially when structural changes of the vessels and metabolic pathways are already present<sup>[7]</sup>.

### **Insulin in the brain**

Insulin receptors are found throughout the brain. The systemic insulin level is increased in most type-1 DM patients due to exogenous insulin treatment. In healthy people insulin is produced in the pancreas, released into the portal circulation and passed on to the liver, where it exerts its prime metabolic effects. When patients with diabetes are treated with exogenous insulin it is absorbed directly into the systemic circulation. This results in 200% increased insulin level in the blood, depending on the injected insulin dose<sup>[7,25]</sup>. Insulin is thought to modulate glucose utilisation in specific brain areas, such as hippocampus with a central role for memory function, and in this way it may affect cognitive functions. Furthermore, brain insulin plays a role in satiety signalling and possible neurodegenerative disorders such as Alzheimer disease<sup>[26]</sup>.

### **Cerebrovascular alterations**

Diabetes is associated with both functional and structural alterations of the cerebral vascular system, which can for example increase the risk of stroke<sup>[7]</sup>. Early changes DM is the reduced neuronal blood flow in the vasa nervorum, which can cause neurophysiological changes, such as endotheliopathy<sup>[19]</sup>. Pathogenically, reduced availability of vasodilating molecules, in particular nitric oxide due to its binding to superoxide forming peroxynitrite, play a central role<sup>[16]</sup>. Secondly, vasoconstrictive factors such as sympathetic tone and angiotensin II levels are increased. Thirdly, increased arterio-venous shunting reduces endoneurial blood flow<sup>[27]</sup>. Later on, structural changes such as pericyte degeneration, capillary membrane thickening and decreased capillary density may occur in the brain<sup>[28]</sup>.

Studies regarding cerebrovascular reactivity in type-1 diabetes patients show that the normal increase in blood flow after administration of dilatory stimuli is impaired in DM patients. The impairment is more severe in patients with diabetes with longer disease duration or who have other complications<sup>[29]</sup>. Cerebral vasoreactivity and accompanying changes in blood flow are important in the preservation of adequate perfusion during abnormal events such as hypotension, hypoxia, hypercapnia and hypoglycaemia, all of which are prevalent in diabetes patients. Loss of these important regulatory mechanism may therefore have detrimental effects on the brain<sup>[7]</sup>.

Besides the above described mechanisms, additional

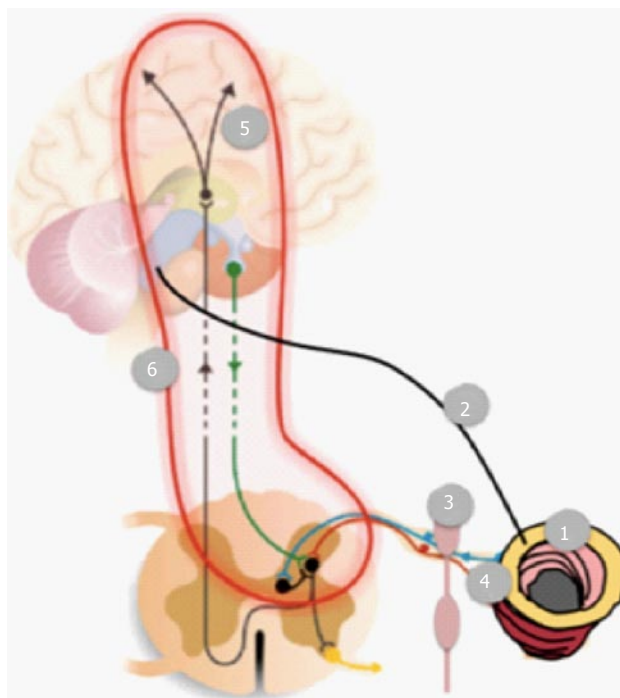
factors, such as disruption of normal endoplasmatic reticulum functioning and the role of autoantibodies may play a role, but will not be further explained here<sup>[27,30,31]</sup>. Finally, it is important to realize that the relative contribution of different factors varies between individuals (depending on characteristics such as sex, age, co-morbidity and stage of disease) and that all these factors interact<sup>[32]</sup>.

## **THE GI SYMPTOMS**

The above theories may lead to better understanding of the brain-gut axis and GI symptoms seen in DM. In the following section the most relevant symptoms will be described.

Fifty percent of DM patients are affected with peripheral neuropathy<sup>[33]</sup>. Diabetic autonomic neuropathy can cause abnormal organ function with symptoms such as urinary incontinence, sexual dysfunction, gastroparesis and nocturnal diarrhoea. Both the parasympathetic and the sympathetic nerves are affected, but in the early stages the vagal nerve seems to be the most vulnerable<sup>[34]</sup>. The vagal nerve has among others a great impact in regulating heart function, which - when damaged - can result in tachycardia and other dysrhythmias. When the sympathetic nerves degenerate the heart rate may fall slightly, but in general diabetes patients with autonomic neuropathy have a resting tachycardia. Furthermore, they may have an impaired adaptability of the heart rate (reduced heart rate variability).

The autonomic nervous system does also have a great impact on GI function. Epidemiological studies have indicated an high prevalence of GI complaints in DM patients<sup>[1-3]</sup>, and as mentioned in the introduction, up to 40% with longstanding diabetes suffer from GI symptoms such as nausea and vomiting<sup>[1,4]</sup>. Abdominal discomfort is also a common symptom, which in severe cases may lead to weight loss and malnutrition. Diarrhoea can be a consequence of abnormal gut motor function, but may also be related to small intestinal bacterial overgrowth and adverse effects to various drugs<sup>[1-3]</sup>. Another prevalent GI symptom is delayed gastric emptying, which can lead to nausea, vomiting and weight loss<sup>[35]</sup>. The peripheral and central neuropathy may in theory also give rise to pain *per se*. Pain is a cardinal symptom of peripheral somatic nerve damage in diabetics, and it is a typical neuropathic pain type. In DM peripheral neuropathy is prevalent and can occur spontaneously or provoked by noxious or non-noxious stimuli<sup>[36]</sup>. As a parallel, in patients with pancreatitis where the visceral nerves are also destroyed, neuropathy may play a major role in the GI symptoms<sup>[37]</sup>. Thus, although speculative, peripheral visceral neuropathy in DM may therefore result in abdominal pain. As central nerve lesions in humans such as in spinal cord injuries and stroke also may give rise to pain, diabetic encephalopathy may also lead to symptoms *per se*<sup>[38,39]</sup>.



**Figure 2** Schematic representation of the possible nerve pathways and mechanisms that theoretical can contribute to gastrointestinal symptoms in diabetics. (1) Vascular and degenerative changes in the enteric nervous system; Autonomic neuropathy affecting (2) the vagal nerve and (3) sympathetic pathways; (4) Affection of visceral (and somatic in case the peritoneum is involved) afferents mediating sensations such as pain; (5) Structural and functional changes in the brain (and spinal cord), together with (6) affection of spino-bulbo-spinal loops.

The relationship between the visceral nerves and the brain is illustrated in Figure 2. The gut changes in DM may however, also be related to changes in the CNS as motility is partly influenced by interaction with several brain areas<sup>[8]</sup>. For example has damage to the dorsal motor nucleus and the paraventricular nucleus of hypothalamus in animals been found of importance for nerves controlling the gut<sup>[9]</sup>, and a pathway including the area postrema, nucleus tractus solitaries and the dorsal motor nucleus of the vagal nerve has been shown to have great impact in controlling gut function including motility<sup>[9]</sup>. Such changes in motility may indirectly lead to symptoms<sup>[38,39]</sup>.

Other symptoms that may relate to diabetic affection of the brain are cognitive dysfunction. This is most marked in patients with an early onset of diabetes. When having type 1 diabetes it is most evident in the domains of psychomotor speed, mental flexibility and general intelligence. Hence, the performance on these domains in patients with diabetes is 30%-40% of that in healthy control subjects<sup>[40]</sup>.

## DIABETES AND THE CNS

### Brain changes in animals

Changes within the brain in DM have been investigated in both animals and humans. Although not the focus of this review, a few selected animal studies are shown

in Table 1. In animals such as rats with long-term streptozotocin-induced diabetes increased abnormality in the neuron cells and blood vessels within the brain have been found. Findings were altered Golgi bodies, mitochondria and endoplasmic reticulum cisterns, concurrent with superoxide dismutase inactivation and aldose reductase accumulation<sup>[41]</sup>. Besides this, changes in dorsal motor and paraventricular nuclei of the hypothalamus have been reported in animal models, both of great importance for controlling the part of the autonomic nerves that innervate the gut<sup>[9]</sup>. Additionally, protein kinase C was found increased, which may cause altered cell functioning as explained in section (The hyperglycaemia hypothesis)<sup>[42]</sup>. However, it is not yet clear to which extend the GI mechanisms are caused by the central neuropathy. Some changes in the gut function may be explained by neurotoxicity of streptozotocin at specific locations in the brain, which could have great impact on the regulation of the gut. When having this in mind the choice of animal model is critical if wrong conclusions are to be avoided and relevance to human disease to be maintained<sup>[9]</sup>. Animal models can inform us of some of the pathophysiological and pathogenic aspects of human disease. An advantage is that they may compare to human cases even though the animal does not necessarily have the same symptoms as in a human condition. On the other hand, major differences in morphology and function between rodent and human brains do exist, making the results of animal studies difficult to translate to humans.

As outlined above, the differences between human and animal brains make it important to focus on human research. Here, methods such as quantitative sensory testing (QST), electrophysiology or imaging can be used to explore functional and structural brain changes in humans with diabetes and GI symptoms, which will be elaborated further on in the next sections.

### QST - methodology

Clinical assessment of the sensory system in patients is affected by, *e.g.*, general malaise, additional pathophysiological influences, or co-medications. QST is a discipline to evoke sensations such as pain under controlled circumstances<sup>[43]</sup>. This is advantageous as it encompasses many of the problems seen in the clinical situation and offers the opportunity to explore the sensory system more objectively. QST can for example determine the vibration sensation threshold and the pain thresholds for cold and warm temperatures by stimulating the skin, but can also be used in the deeper tissues such as muscles and viscera. In many of the studies investigated in this review, the oesophagus has been investigated. The methods are also able to selectively activate different nerve pathways to mimic the clinical situation such as with the multimodal probe that can be used in the oesophagus and rectum<sup>[44]</sup>. Following standardised sensory stimulation there are several ways to measure the response. Psychophysical methods such as intensity ratings



**Table 1** Animal models of diabetes

Ref.	Methods	Results
Bhardwaj <i>et al</i> <sup>[42]</sup>	Signal transduction in brains was investigated in rats with 1-3 mo of induced diabetes	Protein kinase A and C were increased and calcium/calmodulin dependent protein kinase II decreased
Li <i>et al</i> <sup>[81]</sup>	Proteins were extracted from brain tissues of control rats and type 1 diabetic rats	The proteomic identification could be a useful tool for understanding of diabetic encephalopathy mechanisms
Yang <i>et al</i> <sup>[41]</sup>	Rats were randomized into a control and a DM group and neuron and vessel changes were examined with electron microscopy	Increasing abnormality in the neurons and blood vessels were seen that correlated to the length of diabetes
Ramos-Rodriguez <i>et al</i> <sup>[82]</sup>	Brain morphology was analyzed in mice	Hippocampal and cortical atrophy was found as well as cell proliferation and neurogenesis impairment

DM: Diabetes mellitus.

are most frequently used, but they give no detailed information about the brains response that can be explored with electrophysiological or imaging methods. Findings from experimental pain can be used to getting greater knowledge of a disease and pharmacological mechanisms, and clinical pain symptoms may help explaining results from experimental pain studies.

### Findings with QST

QST has been used in several studies to investigate diabetes patients with GI symptoms, as seen in Table 2. The most consistent result to painful gut stimulation in DM was hyposensitivity. Two studies found that DM patients had hyposensitivity to painful sensation evoked in the esophagus<sup>[11,45]</sup>. When using rectosigmoid electrostimulation hyposensitivity to painful stimulation was also found<sup>[4]</sup>. However in another study the authors found no significant differences in sensory or pain threshold to esophageal stimulations between DM patients and healthy controls, and findings are therefore not absolutely consistent<sup>[46]</sup>. In a study where the effect of acute hyperglycaemia was tested, increased gut sensitivity was seen. Hyperglycaemia is thought to affect the nerves, however it had no effect on the sensation in the patients<sup>[47]</sup>. The hyposensitivity seen in DM strongly supports the presence of peripheral neuropathy and it seems to be generalized to several gut segments. Hence, in comparison with healthy controls, Søfteland *et al*<sup>[48]</sup> found evidence of decreased cutaneous and rectal sensations in diabetes patients with sensorimotor neuropathy and increased GI symptoms. Rectal and cutaneous sensitivities were correlated and associated with abnormal heart rate variability supporting that all fibre types were affected. Another QST study in patients with longstanding DM, showed evidence of generalized neuronal damage manifested as sensorimotor, autonomic and central neuropathies, and the degree of peripheral hypoesthesia was associated to both heart rate variability and impaired conditioned pain modulation<sup>[49]</sup>. Pain and other conscious sensations are processed in the brain and traditionally a “bottom-up” model has been suggested; *i.e.*, damage to the peripheral nerve causes central reorganization<sup>[4]</sup>. On the other hand, it cannot be excluded that spinal, brainstem or brain changes due to central neuropathy alone may contribute to the

generalized sensory changes.

QST can also be used to evoke referred pain. This is partly due to convergence between visceral and somatic afferents in the dorsal horn of the spinal cord, and any central hyperexcitability will increase the size of the referred pain area<sup>[50-53]</sup>. As increases in the referred pain areas were seen following stimulation of the upper gut in DM, this indicates a widely distribution of central sensitization.

### Electroencephalography - methodology

EEG is the recording of electrical activity on the scalp produced by activation of neurons in the brain. The activity can basically be recorded as either evoked potentials following an external stimulus, or in the resting state<sup>[54]</sup>. In both types of recordings, the EEG can be used to study normal pain processing and to identify alterations of pain processing in different patient groups<sup>[54]</sup>. Most studies used evoked brain potentials (EPs), the concept illustrated in Figure 3. The advantage of EPs is that they can detect neuronal activity with very high temporal resolution, thereby making analysis of the primary sensory-specific upstream activation of brain centres possible. This is of major importance as these activations take place within the first 200 ms after stimulation of the periphery<sup>[54]</sup>. Compared with methods based on hemodynamic and metabolic changes (positron emission tomografi and functional MRI) EPs have a better time resolution (ms) and with the newest models the corresponding brain sources can be modelled with a spatial resolution of a few mm<sup>[54,55]</sup>.

Electrical stimulation is often used to activate the nervous system but it is unspecific as it bypasses peripheral receptors and depolarizes all types of nerve fibres. In the gut electrical stimulation primarily activates Aδ fibers although the majority are unmyelinated C-fibres, but the electrical stimulus is still to be favored due to the high temporal accuracy. Recently methods such as rapid balloon distension, which is a more natural stimulus, have been developed, but they have never been used in patients with diabetes<sup>[56]</sup>. Furthermore, EPs have been recorded following non-visceral sources such as auditory, visual and somatosensory stimuli, and abnormal response in the brain has been observed in patients with type 1 and 2 diabetes<sup>[7]</sup>. There has been

**Table 2 Findings with quantitative sensory testing and evoked brain potentials in patients with diabetes and gastrointestinal symptoms**

Ref.	Method	Results
Frøkjær <i>et al</i> <sup>[11]</sup>	12 healthy controls and 12 type-1 diabetes patients with proven autonomic neuropathy and severe GI symptoms had their sensitivity to stimulations in the oesophagus and duodenum assessed	A 46% increase in the somatic referred pain areas, indicating central hyper excitability. The results also indicated that the sensory nerves in the GI tract were widely affected. Furthermore it is suggested that future targets in the treatment of GI symptoms in DM patients could be based on modulation of the central nervous system excitability
Frøkjær <i>et al</i> <sup>[63]</sup>	14 type-1 diabetes patients with autonomic neuropathy and GI symptoms and 15 healthy volunteers had their sensitivity to electrical oesophageal and median nerve stimulations assessed by using an euglycemic-hyperinsulinemic clamp. The EPs were also collected	GI symptoms correlated with characteristics of brain potentials in the DM patients. These results indicate a change in peripheral visceral nerves as well as in the central nervous system
Frøkjær <i>et al</i> <sup>[47]</sup>	Evoked potentials to oesophageal and median nerve stimulations were recorded in 14 type-1 diabetes patients with GI symptoms	The study concluded that acute hyperglycaemia had no effect on the brain activation of visceral and somatic stimulations
Frøkjær <i>et al</i> <sup>[62]</sup>	15 healthy volunteers and 14 type-1-diabetes patients with autonomic neuropathy and related GI symptoms had their EPs recorded following painful oesophageal electrical stimulation	Evidence of altered central processing to visceral stimulation in diabetes was found. Compared to controls, the patients with diabetes had a posterior shift of the electrical sources in the anterior cingulate cortex, and additional sources close to the posterior insula and in medial frontal gyrus
Frøkjær <i>et al</i> <sup>[45]</sup>	Ultrasound monitored oesophageal distension was used to study 17 patients with longstanding DM and GI symptoms and 13 healthy controls	The reduced sensitivity was associated with the presence of peripheral neuropathy. This indicates a coexisting change within the visceral and somatic neuropathy
Brock <i>et al</i> <sup>[64]</sup>	14 type-1 diabetes patients with diabetes autonomy neuropathy and 15 healthy volunteers underwent multichannel EEG during painful electrical stimulation of the lower esophagus	Central neuroplastic changes within DM patients were found in the insular region, and it was suggested that the GI symptoms are due to the abnormal insular processing
Lelic <i>et al</i> <sup>[46]</sup>	Electrical stimulation of the rectum was done in 12 healthy controls and 12 type 1 diabetes patients with GI symptoms while having their EPs recorded	Changes in the cingulate-operculum brain network were found in DM patients with GI symptoms. Changes could serve as a biomarker of disturbed sensory visceral processing and GI symptoms in patients with diabetes
Brock <i>et al</i> <sup>[4]</sup>	15 healthy volunteers and 15 diabetes patients with GI symptoms and clinical suspicion of autonomic neuropathy were included. Electrical source analysis to painful recto-sigmoid electrostimulations was modelled	Patients with autonomic neuropathy and GI symptoms had evidence for altered brain activation and dysregulation of the central regulation of the autonomic nervous system, which could explain appearance and persistence of upper GI symptoms
Søfteland <i>et al</i> <sup>[48]</sup>	16 healthy controls and 20 DM patients with sensorimotor polyneuropathy had their heart rate variability and peripheral tactile thresholds recorded and underwent a cold-pressor-test	The patients in this study suffered from generalized polyneuropathy evident as autonomic neuropathy, peripheral hypoesthesia and central changes manifested as impaired conditioned pain modulation
Lelic <i>et al</i> <sup>[61]</sup>	EPs to electrical esophageal stimulation were achieved in 23 diabetes patients with upper GI symptoms and 27 healthy controls. Network analysis between active sources were performed	There was a reorganisation in the opercular cortex, which was correlated with GI symptoms. It was proposing that the changes in the operculo-cingulate cortex could help explain the development and maintenance of GI symptoms in diabetes patients

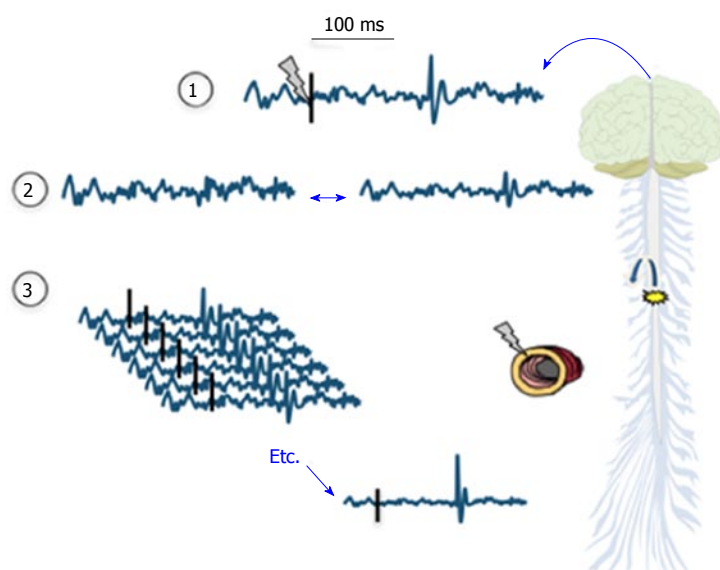
EPs: Evoked brain potentials; GI: Gastrointestinal; EEG: Electroencephalography; DM: Diabetes mellitus.

focus on the so-called late responses as reflected in the P300 wave of somatosensory stimuli, where the latency was increased in DM patients, and it has been hypothesized that evoked potential changes may appear before cognitive dysfunction develops<sup>[57]</sup>. The resting EEG has also been used to investigate the brain in patients with diabetes. This has revealed differences in brain connectivity and information flow, but the changes were not related to the evoked potentials or cognitive functions, and hence assess other functions of the brain<sup>[57,58]</sup>. More sophisticated analyses have confirmed that resting EEG synchronization and complexity is also related to cognitive function and blood glucose level<sup>[59,60]</sup>. However, resting EEG has not been used to explore neuropathy or GI changes and hence it will not be elaborated further in this review. Recently the neurophysiological changes using EPs to visceral organs

in patients with GI symptoms have been investigated and these will be presented below.

### Findings with electroencephalography

Studies with EPs have shown evidence of altered central processing to visceral stimulation in diabetes patients with GI symptoms and the findings were repeated in several studies<sup>[61,62]</sup>. Table 2 shows an overview of the different studies. Using evoked potentials to stimulation of recto-sigmoid and esophagus the latencies of the EPs at vertex were increased and amplitudes reduced in DM patients with major variability between patients<sup>[62]</sup>. In recent studies where multiple channels (64-128) were recorded the authors were able to model the corresponding brain sources<sup>[2]</sup>. Compared with controls, the patients had a posterior shift of the electrical sources in the anterior cingulate cortex to oesophageal stimulation,



**Figure 3** Evoked potentials are recorded following a peripheral stimulus as indicated with the grey “lightning” and ideally a corresponding activity can be seen following 80-90 ms as an evoked potential. However, as illustrated in (2) the amplitudes of the evoked potential tend to be low and often comparable to the amplitudes of spontaneous electroencephalogram. In order to decode the evoked potentials from the background electroencephalographic activity and noise, signal averaging is necessary as illustrated in (3). Provided enough of recorded trials, the evoked potentials become bigger in amplitude and therefore visible and the random background activity cancel out. Then, the EP latencies and amplitudes of the peaks can be analyzed by visual inspection. When many (64-128) electrodes are used the corresponding brain sources can be computed based on the surface electroencephalographic recordings. EP: Evoked brain potential.

and additional sources close to the medial frontal gyrus and posterior insula<sup>[62]</sup>. Another study conducted with a similar method found that the GI symptoms correlated with characteristics of brain potentials in the diabetes patients<sup>[63]</sup>. Furthermore a study conducted by the same group showed that DM patients had an anterior shift in insular and cingulate source localizations compared to healthy controls<sup>[64]</sup>. As the insula is considered one of the main centres from where the upstream activation of visceral information is controlled, this may have major importance for our understanding of GI symptoms in DM. In fact the research showed that the more GI symptoms (vomiting, nausea, early satiety, diarrhoea, abdominal pain and/or constipation) the patients experienced, the more anteriorly the insular source was located<sup>[64]</sup>.

GI symptoms and their development and maintenance were also found to correlate with reorganization within the opercular cortex localised between the insula and secondary somatosensory cortex<sup>[61]</sup>. Another study explored the communication between different brain regions. The changes in networks were correlated to severity of upper GI symptoms and life quality. It was concluded that changes in the networks could also serve as a biomarker of disturbed sensory visceral processing and GI symptoms in patients with diabetes<sup>[46]</sup>.

### Imaging methods

Another method is brain imaging where magnetic resonance (MR) is the dominating method. The contrast between grey and white matter in MRI makes it the optimal choice for many conditions of the CNS including demyelination. A specific way of analysing MR images is volumetry where the total brain volume is determined by summing the grey and white matter pixels, then multiplying by the voxel dimension. With this method, a more precise estimate of atrophy can be accessed<sup>[65]</sup>. Another MRI method used in some of the studies in this review is functional magnetic

resonance (fMRI) with measurement of blood oxygen level dependent contrasts (BOLD). It measures brain activity by detecting changes in blood oxygenation. It infers regional changes in brain activity, and thereby it reflects whether a specific brain region is engaged to a time-linked neurobehavioral or neurocognitive task. It has been of great importance in mapping regions in the brain linked to specific functions<sup>[66]</sup>. More specifically it is a great method to examine the superficial layers of the brain because of the excellent spatial resolution (2-5 mm), but limitations are seen in the deeper structures, such as brainstem and thalamus because of pulsation artefacts. fMRI has the possibility to take individual characteristics and anatomy into account, which can be a major advantage. Furthermore, fMRI operates in a non-radioactive and non-invasive radioactive environment, allowing subjects to be studied repetitively<sup>[54]</sup>. Though there are many advantages of fMRI there are some limitations, for example the fMRI is clearly inferior in temporal resolution compared to EEG/MEG, meaning that in pain studies fMRI is not a specific tool for investigating the primary neuronal activity directly related to the painful stimuli. Additionally, the fMRI activity to GI stimulation is not stimulus-specific. Hence, anticipation of stimuli can trigger similar activity and repeated activation can result in habituation<sup>[54]</sup>. Another imaging method is diffusion tensor imaging (DTI)<sup>[67]</sup>. It measures the directionality and magnitude of water diffusion in tissues. The mechanism behind neurostructural changes is not clear but it is the general belief that the integrity of axonal membrane and myelin sheaths is reflected by restriction of diffusion perpendicular to the fibres. The intra-axonal structures, such as microtubules, are thought reflected by diffusion parallel to the fibres. DTI measures the magnitude [described by the apparent diffusion coefficient (ADC)] and directionality [described as fractional anisotropy (FA)] of water diffusion in tissues. Reduced ADC values are seen in variety of CNS insults such as stroke and

trauma, whereas reduced FA is often seen in schizophrenia, Alzheimer's disease and depression<sup>[68]</sup>.

The last method relevant for this review is the arterial spin labelling, which allows the measurement of whole brain blood flow in absolute units through the use of magnetically labelled endogenous water in blood acting as a diffusible tracer<sup>[54]</sup>. This method makes it possible to track the temporal dynamics of the neural activation induced by pain. It is a suitable method to measure the brain's response to tonic stimuli and symptoms since it is more sensitive than fMRI to changes in neural activation when stimulus duration exceeds 1 min<sup>[54]</sup>.

### Imaging findings

Brain MRI conducted in diabetics has been used in different contexts. Most studies have been done in non-selected patients - *i.e.*, without GI symptoms. In type 2 DM atrophy of the brain is mostly found in areas responsible for verbal, visual memory, executive functioning and information processing, and this may link to an increased risk for developing dementia<sup>[40]</sup>. There has also been found cortical atrophy in type-2 diabetes patients, resembling patterns in preclinical Alzheimer's disease<sup>[69]</sup>. Cerebral dysfunction has been convincingly shown in patients with type 1 and 2 diabetes, but few neuroradiological studies have been conducted in patients with autonomic and GI symptoms<sup>[70]</sup>. Due to the sparse imaging research with focus on GI problems and diabetes this review has included few studies regarding general brain changes, as well as studies where brain changes, were comparable to those in animal studies with a correlate to GI symptoms. Generally, as shown in Table 3 structural changes with central atrophy has been reported in patients with DM. One study on type 2 diabetes patients found cortical and subcortical atrophy involving frontal and temporal brain regions, with diminished vasoreactivity and regional cerebral perfusion<sup>[71]</sup>. This supports that uncontrolled diabetes may further contribute to hypoperfusion and atrophy. More specifically MR has also been used to discover a larger lateral ventricular volume with larger white matter lesion. However, no white matter volume difference was found, which is opposite the grey matter shrinkage in the DM patients<sup>[72]</sup>. In another study with MR imaging the focus was atrophy and aging, and in midlife diabetes was associated with subcortical infarctions. More specifically a reduced hippocampal volume, whole brain volume atrophy, and mild cognitive impairment were found<sup>[73]</sup>. The reduced hippocampal volume has been confirmed in different studies including animal studies<sup>[9,69,73,74]</sup>. On the other hand a study that looked into the macrostructural brain alterations found no overall alterations with standard evaluations of the images, and it may be that the macrostructural brain changes are limited in well-treated type-1 diabetes patients<sup>[75]</sup>. It has also been suggested that the

radiological appearance of the brain in patients with diabetes resembles that of normal ageing, but appears to develop at a younger age than in healthy controls<sup>[7]</sup>. In one study investigating the effect of diabetes on brain atrophy and cognitive impairment, pathological findings were only significant in women. The differences between the genders were an unexpected finding and need to be investigated in more detail<sup>[72]</sup>.

Changes in cerebral blood flow in type 2 diabetes have also been investigated with arterial spin labelling<sup>[71]</sup>. Type 2 diabetes were associated with cortical and subcortical atrophy involving frontal and temporal brain regions and with diminished vasoreactivity and regional cerebral perfusion. Additionally the same study found that uncontrolled diabetes might further contribute to hypoperfusion and atrophy.

In patients with DM and GI symptoms a study used cortical volumetry and found reduced cortical thickness of the postcentral and superior parietal gyrus in patients. Those with peripheral neuropathy showed reduction in right postcentral gyrus cortical thickness compared to patients without neuropathy<sup>[74]</sup>. DTI has also been used to investigate more subtle changes in the brain. More specifically a study found that patients with long-standing DM and GI symptoms have microstructural changes in brain areas involved in visceral sensory processing. This could be related to DM-induced brain changes specific for the gut, although, *e.g.*, insular changes may also be important in dysregulation of other functions<sup>[68]</sup>. The microstructural changes were for some areas correlated to GI parameters such as bloating and presence of gastroparesis, together with other autonomic dysfunctions and therefore may be involved in the pathogenesis of GI symptoms. However, even though the few studies in MR are consistent and microstructural alterations were found in diabetes patients with GI symptoms, they still need confirmation in other studies.

## POTENTIAL EFFECTS OF ANTIDIABETIC TREATMENT

Many antidiabetics can potentially protect against harmful changes in the CNS. Hence, according to the pathophysiology section above improved blood sugar control and sparing of exogenous insulin will likely result in less neuronal damage. Furthermore, new antidiabetics such as the incretin hormone GLP-1 may be beneficial. Despite its insulinotropic actions it has many unexplored extra-pancreatic effects. Hence, GLP-1 receptors are, in addition to the pancreas, found in the heart, lungs, kidneys and elsewhere in the GI tract, and its function in many of these locations is not yet fully understood<sup>[76]</sup>. In the CNS it primarily affects stimulation of glucose-dependent insulin secretion<sup>[77]</sup> and inhibition of glucagon secretion<sup>[78]</sup>. Interestingly, GLP-1 also acts as a neuropeptide with direct effect on regulation of vagal activity, consequently modulating



**Table 3** Imaging findings in patients with diabetes

Ref.	Method	Results
Jongen <i>et al</i> <sup>[72]</sup>	MR images of 99 DM patients and 46 controls	Larger lateral ventricular volume with white matter lesion and smaller great matter volume was seen in the diabetes patients. The effect of diabetes on brain atrophy where only significant in women
Kodl <i>et al</i> <sup>[83]</sup>	25 type-1 diabetes patients and controls were scanned with a diffusion tensor imaging protocol	White matter microstructural deficits in patients with longstanding diabetes type-1 were found. The deficits correlated with the neurocognitive tests
Last <i>et al</i> <sup>[71]</sup>	Cerebral blood flow was examined in 26 diabetes patients and 25 controls using continuous arterial spin labeling imaging during baseline, CO <sub>2</sub> rebreathing and hyperventilation	Type-2 diabetes was associated with cortical and subcortical atrophy involving frontal and temporal brain regions and with diminished vasoreactivity and regional cerebral perfusion. Uncontrolled diabetes may further contribute to hypoperfusion and atrophy
Kamiyama <i>et al</i> <sup>[74]</sup>	Voxel-based morphometric analysis was performed on 28 diabetes patients and 28 controls	Diabetes patients had hippocampal region atrophy and whole-brain atrophy
Northham <i>et al</i> <sup>[84]</sup>	MRI and IQ test were performed on 106 type-1 diabetes patients and 75 control subjects at baseline and then a 12-yr follow-up	DM subjects had lower verbal and full scale IQs, a decreased gray matter in bilateral thalami and right parahippocampal gyrus and insular cortex. White matter was decreased in bilateral parahippocampus, left temporal lobe, and middle frontal area
van Elderen <i>et al</i> <sup>[32]</sup>	Cognitive function test and MRI was conducted on 438 control subjects and 89 DM patients aged 70-82 yr	Elderly DM patients have accelerated progression of brain atrophy with significant consequences in cognition compared to the control subjects
Frøkjær <i>et al</i> <sup>[68]</sup>	MR scanning was performed in 23 controls and 26 patients with DM and GI symptoms and diffusion tensor imaging was performed	Diabetes patients had microstructural changes in brain areas involved in visceral sensory processing. This could be related to generalized DM-induced brain changes
Rosebud <i>et al</i> <sup>[73]</sup>	MRI on 51437 subjects including 214 with diabetes was performed	Midlife diabetes was associated with subcortical infarctions. Reduced hippocampal volume, whole brain volume and mild cognitive impairment were registered in diabetes patients
Frøkjær <i>et al</i> <sup>[75]</sup>	20 healthy controls and 15 patients with longstanding type 1 diabetes mellitus were scanned and cortical thickness was assessed based on a cortical segmentation method	Reduced cortical thickness of superior parietal and postcentral gyrus. No overall macrostructural brain alterations were detected, but the authors concluded that cortical thinning involving sensory related areas might be important in diabetes

MR: Magnetic resonance; MRI: Magnetic resonance imaging; GI: Gastrointestinal; IQ: Intelligence quotient; DM: Diabetes mellitus.

the homeostatic regulation of the gut<sup>[78]</sup>. Recently, potential neuroprotective function through activation of the GLP-1 axis has received more attention<sup>[79]</sup>, and GLP-1 expression has been identified in neurons of the nodose ganglion including sensory afferents critical to many autonomic reflexes. Furthermore, diabetes patients with autonomous neuropathy were shown to have altered incretin effect as compared to patients without neuropathy<sup>[80]</sup>. Therefore - although it is not recommended to use GLP-1 agonists in patients with diabetic gastroparesis - such drugs may be neuroprotective and human studies are highly warranted.

## CONCLUSION

Investigation of the existing literature on diabetes patients with GI symptoms indicates the presence of structural and functional brain changes. This association was most consistent in EEG studies, but this may relate to the greater amount of papers using this technique. Especially changes in the insula seemed to correlate with GI symptoms. In fact there was evidence that the more GI symptoms the patients experienced, the more changes in insular source was seen, and communications between the insula and other brain regions were malfunctioning. The EEG was often used in combination with QST, which mainly indicated

visceral hyposensitivity in the patients with diabetes and GI symptoms. Imaging studies on diabetes patients with GI symptoms indicated microstructural changes in brain areas involved in visceral sensory processing. Due to these findings, future targets in treatment of GI symptoms may be based on modulation of the CNS reorganisation, either pharmacologically or with afferent nerve stimulation.

## REFERENCES

- 1 **Bytzer P**, Talley NJ, Leemon M, Young LJ, Jones MP, Horowitz M. Prevalence of gastrointestinal symptoms associated with diabetes mellitus: a population-based survey of 15,000 adults. *Arch Intern Med* 2001; **161**: 1989-1996 [PMID: 11525701]
- 2 **Ricci JA**, Siddique R, Stewart WF, Sandler RS, Sloan S, Farup CE. Upper gastrointestinal symptoms in a U.S. national sample of adults with diabetes. *Scand J Gastroenterol* 2000; **35**: 152-159 [PMID: 10720112]
- 3 **Ko GT**, Chan WB, Chan JC, Tsang LW, Cockram CS. Gastrointestinal symptoms in Chinese patients with Type 2 diabetes mellitus. *Diabet Med* 1999; **16**: 670-674 [PMID: 10477212]
- 4 **Brock C**, Søfteland E, Gunterberg V, Frøkjær JB, Lelic D, Brock B, Dimcevski G, Gregersen H, Simrén M, Drewes AM. Diabetic autonomic neuropathy affects symptom generation and brain-gut axis. *Diabetes Care* 2013; **36**: 3698-3705 [PMID: 24026548 DOI: 10.2337/dc13-0347]
- 5 **Koppen BM**, Stanton BA. *Berne & Levy Physiology*, 6th Updated Edition, with Student Consult Online Access 6th Edition. Mosby, 2010: 693
- 6 **Deguchi T**, Nishio Y, Takashima H. Diabetes mellitus and

- autoimmune neuropathy. *Brain Nerve* 2014; **66**: 135-147 [PMID: 24523312]
- 7 **Brands AM**, Kessels RP, de Haan EH, Kappelle LJ, Biessels GJ. Cerebral dysfunction in type 1 diabetes: effects of insulin, vascular risk factors and blood-glucose levels. *Eur J Pharmacol* 2004; **490**: 159-168 [PMID: 15094082 DOI: 10.1016/j.ejphar.2004.02.053]
- 8 **Browning KN**, Travagli RA. Central nervous system control of gastrointestinal motility and secretion and modulation of gastrointestinal functions. *Compr Physiol* 2014; **4**: 1339-1368 [PMID: 25428846 DOI: 10.1002/cphy.c130055]
- 9 **Horowitz M**, Samsom M. Gastrointestinal Function in Diabetes Mellitus. Wiley Online Library, 2004: 44-45, 67 [DOI: 10.1002/0470013877]
- 10 **Frøkjær JB**, Ejlskjær N, Rask P, Andersen SD, Gregersen H, Drewes AM, Funch-Jensen P. Central neuronal mechanisms of gastric electrical stimulation in diabetic gastroparesis. *Scand J Gastroenterol* 2008; **43**: 1066-1075 [PMID: 18609155 DOI: 10.1080/00365520802028221]
- 11 **Frøkjær JB**, Andersen SD, Ejlskjær N, Funch-Jensen P, Arendt-Nielsen L, Gregersen H, Drewes AM. Gut sensations in diabetic autonomic neuropathy. *Pain* 2007; **131**: 320-329 [PMID: 17521809 DOI: 10.1016/j.pain.2007.04.009]
- 12 **Apkarian AV**, Sosa Y, Sonty S, Levy RM, Harden RN, Parrish TB, Gitelman DR. Chronic back pain is associated with decreased prefrontal and thalamic gray matter density. *J Neurosci* 2004; **24**: 10410-10415 [PMID: 15548656 DOI: 10.1523/jneurosci.2541-04.2004]
- 13 **Selvarajah D**, Wilkinson ID, Davies J, Gandhi R, Tesfaye S. Central nervous system involvement in diabetic neuropathy. *Curr Diab Rep* 2011; **11**: 310-322 [PMID: 21667355 DOI: 10.1007/s11892-011-0205-z]
- 14 **Tomlinson DR**, Gardiner NJ. Glucose neurotoxicity. *Nat Rev Neurosci* 2008; **9**: 36-45 [PMID: 18094705]
- 15 **Chowdhury SK**, Smith DR, Fernyhough P. The role of aberrant mitochondrial bioenergetics in diabetic neuropathy. *Neurobiol Dis* 2013; **51**: 56-65 [PMID: 22446165 DOI: 10.1016/j.nbd.2012.03.016]
- 16 **Hosseini A**, Abdollahi M. Diabetic neuropathy and oxidative stress: therapeutic perspectives. *Oxid Med Cell Longev* 2013; **2013**: 168039 [PMID: 23738033 DOI: 10.1155/2013/168039]
- 17 **Obrosova IG**, Drel VR, Pacher P, Illytska O, Wang ZQ, Stevens MJ, Yorek MA. Oxidative-nitrosative stress and poly(ADP-ribose) polymerase (PARP) activation in experimental diabetic neuropathy: the relation is revisited. *Diabetes* 2005; **54**: 3435-3441 [PMID: 16306359]
- 18 **Edwards JL**, Vincent AM, Cheng HT, Feldman EL. Diabetic neuropathy: mechanisms to management. *Pharmacol Ther* 2008; **120**: 1-34 [PMID: 18616962 DOI: 10.1016/j.pharmthera.2008.05.005]
- 19 **Sytze Van Dam P**, Cotter MA, Bravenboer B, Cameron NE. Pathogenesis of diabetic neuropathy: focus on neurovascular mechanisms. *Eur J Pharmacol* 2013; **719**: 180-186 [PMID: 23872412 DOI: 10.1016/j.ejphar.2013.07.017]
- 20 **Callaghan BC**, Little AA, Feldman EL, Hughes RA. Enhanced glucose control for preventing and treating diabetic neuropathy. *Cochrane Database Syst Rev* 2012; **6**: CD007543 [PMID: 22696371 DOI: 10.1002/14651858.CD007543.pub2]
- 21 **Linn T**, Ortac K, Laube H, Federlin K. Intensive therapy in adult insulin-dependent diabetes mellitus is associated with improved insulin sensitivity and reserve: a randomized, controlled, prospective study over 5 years in newly diagnosed patients. *Metabolism* 1996; **45**: 1508-1513 [PMID: 8969284]
- 22 The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. *N Engl J Med* 1993; **329**: 977-986 [PMID: 8366922 DOI: 10.1056/nejm199309303291401]
- 23 **Perros P**, Deary IJ, Sellar RJ, Best JJ, Frier BM. Brain abnormalities demonstrated by magnetic resonance imaging in adult IDDM patients with and without a history of recurrent severe hypoglycemia. *Diabetes Care* 1997; **20**: 1013-1018 [PMID: 9167117]
- 24 **Chabriat H**, Sachon C, Levasseur M, Grimaldi A, Pappata S, Rougemont D, Masure MC, De Recondo A, Samson Y. Brain metabolism after recurrent insulin induced hypoglycaemic episodes: a PET study. *J Neurol Neurosurg Psychiatry* 1994; **57**: 1360-1365 [PMID: 7964812]
- 25 **Nijs HG**, Radder JK, Poorthuis BJ, Krans HM. Insulin resistance in type 1 (insulin-dependent) diabetes: dissimilarities for glucose and intermediary metabolites. *Diabetes Res* 1990; **15**: 15-19 [PMID: 2132195]
- 26 **Kleinridders A**, Ferris HA, Cai W, Kahn CR. Insulin action in brain regulates systemic metabolism and brain function. *Diabetes* 2014; **63**: 2232-2243 [PMID: 24931034 DOI: 10.2337/db14-0568]
- 27 **Cameron NE**, Eaton SE, Cotter MA, Tesfaye S. Vascular factors and metabolic interactions in the pathogenesis of diabetic neuropathy. *Diabetologia* 2001; **44**: 1973-1988 [PMID: 11719828 DOI: 10.1007/s001250100001]
- 28 **Johnson PC**, Brendel K, Meezan E. Thickened cerebral cortical capillary basement membranes in diabetics. *Arch Pathol Lab Med* 1982; **106**: 214-217 [PMID: 6896132]
- 29 **Fülesdi B**, Limburg M, Bereczki D, Michels RP, Neuwirth G, Legemate D, Valikovic A, Csiba L. Impairment of cerebrovascular reactivity in long-term type 1 diabetes. *Diabetes* 1997; **46**: 1840-1845 [PMID: 9356034]
- 30 **Cameron NE**. Role of endoplasmic reticulum stress in diabetic neuropathy. *Diabetes* 2013; **62**: 696-697 [PMID: 23431013 DOI: 10.2337/db12-1469]
- 31 **Knopp M**, Rajabally YA. Common and less common peripheral nerve disorders associated with diabetes. *Curr Diabetes Rev* 2012; **8**: 229-236 [PMID: 22283678]
- 32 **van Elderen SG**, de Roos A, de Craen AJ, Westendorp RG, Blauw GJ, Jukema JW, Bollen EL, Middelkoop HA, van Buchem MA, van der Grond J. Progression of brain atrophy and cognitive decline in diabetes mellitus: a 3-year follow-up. *Neurology* 2010; **75**: 997-1002 [PMID: 20837967 DOI: 10.1212/WNL.0b013e3181f25f06]
- 33 **Tesfaye S**, Boulton AJ, Dyck PJ, Freeman R, Horowitz M, Kempler P, Lauria G, Malik RA, Spallone V, Vinik A, Bernardi L, Valensi P. Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. *Diabetes Care* 2010; **33**: 2285-2293 [PMID: 20876709 DOI: 10.2337/dc10-1303]
- 34 **Schönauer M**, Thomas A, Mörbach S, Niebauer J, Schönauer U, Thiele H. Cardiac autonomic diabetic neuropathy. *Diab Vasc Dis Res* 2008; **5**: 336-344 [PMID: 18958844 DOI: 10.3132/dvdr.2008.047]
- 35 **Olausson EA**, Brock C, Drewes AM, Grundin H, Isaksson M, Stotzer P, Abrahamsson H, Attvall S, Simrén M. Measurement of gastric emptying by radiopaque markers in patients with diabetes: correlation with scintigraphy and upper gastrointestinal symptoms. *Neurogastroenterol Motil* 2013; **25**: e224-e232 [PMID: 23316944 DOI: 10.1111/nmo.12075]
- 36 **Ktirji B**, Koontz D. Disorders of Peripheral Nerves. *Bradley's Neurology in Clinical Practice*, 2013-2014
- 37 **Poulsen JL**, Olesen SS, Malver LP, Frøkjær JB, Drewes AM. Pain and chronic pancreatitis: a complex interplay of multiple mechanisms. *World J Gastroenterol* 2013; **19**: 7282-7291 [PMID: 24259959 DOI: 10.3748/wjg.v19.i42.7282]
- 38 **Drewes AM**, Andreassen A, Poulsen LH. Valproate for treatment of chronic central pain after spinal cord injury. A double-blind cross-over study. *Paraplegia* 1994; **32**: 565-569 [PMID: 7970862 DOI: 10.1038/sc.1994.89]
- 39 **Hansen AP**, Marcussen NS, Klit H, Andersen G, Finnerup NB, Jensen TS. Pain following stroke: a prospective study. *Eur J Pain* 2012; **16**: 1128-1136 [PMID: 22407963 DOI: 10.1002/j.1532-2149.2012.00123.x]
- 40 **Biessels GJ**, Reijmer YD. Brain changes underlying cognitive dysfunction in diabetes: what can we learn from MRI? *Diabetes* 2014; **63**: 2244-2252 [PMID: 24931032 DOI: 10.2337/db14-0348]
- 41 **Yang H**, Fan S, Song D, Wang Z, Ma S, Li S, Li X, Xu M, Xu M, Wang X. Long-term streptozotocin-induced diabetes in rats leads to severe damage of brain blood vessels and neurons via enhanced

- oxidative stress. *Mol Med Rep* 2013; **7**: 431-440 [PMID: 23232924 DOI: 10.3892/mmr.2012.1227]
- 42 **Bhardwaj SK**, Sandhu SK, Sharma P, Kaur G. Impact of diabetes on CNS: role of signal transduction cascade. *Brain Res Bull* 1999; **49**: 155-162 [PMID: 10435778]
  - 43 **Olesen AE**, Andresen T, Staahl C, Drewes AM. Human experimental pain models for assessing the therapeutic efficacy of analgesic drugs. *Pharmacol Rev* 2012; **64**: 722-779 [PMID: 22722894 DOI: 10.1124/pr.111.005447]
  - 44 **Drewes AM**, Schipper KP, Dimcevski G, Petersen P, Andersen OK, Gregersen H, Arendt-Nielsen L. Multimodal assessment of pain in the esophagus: a new experimental model. *Am J Physiol Gastrointest Liver Physiol* 2002; **283**: G95-103 [PMID: 12065296 DOI: 10.1152/ajpgi.00496.2001]
  - 45 **Frøkjær JB**, Brock C, Brun J, Simrén M, Dimcevski G, Funch-Jensen P, Drewes AM, Gregersen H. Esophageal distension parameters as potential biomarkers of impaired gastrointestinal function in diabetes patients. *Neurogastroenterol Motil* 2012; **24**: 1016-e544 [PMID: 22738347 DOI: 10.1111/j.1365-2982.2012.01966.x]
  - 46 **Lelic D**, Brock C, Søfteland E, Frøkjær JB, Andresen T, Simrén M, Drewes AM. Brain networks encoding rectal sensation in type 1 diabetes. *Neuroscience* 2013; **237**: 96-105 [PMID: 23384609 DOI: 10.1016/j.neuroscience.2013.01.049]
  - 47 **Frøkjær JB**, Søfteland E, Graversen C, Dimcevski G, Drewes AM. Effect of acute hyperglycaemia on sensory processing in diabetic autonomic neuropathy. *Eur J Clin Invest* 2010; **40**: 883-886 [PMID: 20624170 DOI: 10.1111/j.1365-2362.2010.02335.x]
  - 48 **Søfteland E**, Brock C, Frøkjær JB, Brøgger J, Madácsy L, Gilja OH, Arendt-Nielsen L, Simrén M, Drewes AM, Dimcevski G. Association between visceral, cardiac and sensorimotor polyneuropathies in diabetes mellitus. *J Diabetes Complications* 2014; **28**: 370-377 [PMID: 24355661 DOI: 10.1016/j.jdiacomp.2013.10.009]
  - 49 **Brock C**, Søfteland E, Frøkjær JB, Drewes AM, Nielsen LA. Associations between Sensorimotor, Autonomic and Central Neuropathies in Diabetes Mellitus. *J Diabetes Metab* 2014; **5**: 390 [DOI: 10.4172/2155-6156.1000390]
  - 50 **Drewes AM**, Reddy H, Pedersen J, Funch-Jensen P, Gregersen H, Arendt-Nielsen L. Multimodal pain stimulations in patients with grade B oesophagitis. *Gut* 2006; **55**: 926-932 [PMID: 16091554 DOI: 10.1136/gut.2005.067769]
  - 51 **Reddy H**, Staahl C, Arendt-Nielsen L, Gregersen H, Drewes AM, Funch-Jensen P. Sensory and biomechanical properties of the esophagus in non-erosive reflux disease. *Scand J Gastroenterol* 2007; **42**: 432-440 [PMID: 17454852 DOI: 10.1080/00365520600973099]
  - 52 **Drewes AM**, Petersen P, Rössel P, Gao C, Hansen JB, Arendt-Nielsen L. Sensitivity and distensibility of the rectum and sigmoid colon in patients with irritable bowel syndrome. *Scand J Gastroenterol* 2001; **36**: 827-832 [PMID: 11495078]
  - 53 **Dimcevski G**, Staahl C, Andersen SD, Thorsgaard N, Funch-Jensen P, Arendt-Nielsen L, Drewes AM. Assessment of experimental pain from skin, muscle, and esophagus in patients with chronic pancreatitis. *Pancreas* 2007; **35**: 22-29 [PMID: 17575541 DOI: 10.1097/mpa.0b013e31805c1762]
  - 54 **Frøkjær JB**, Olesen SS, Graversen C, Andresen T, Lelic D, Drewes AM. Neuroimaging of the human visceral pain system-A methodological review. *Scand J Pain* 2011; **2**: 95-104 [DOI: 10.1016/j.sjpain.2011.02.006]
  - 55 **Lelic D**, Gratkowski M, Valeriani M, Arendt-Nielsen L, Drewes AM. Inverse modeling on decomposed electroencephalographic data: a way forward? *J Clin Neurophysiol* 2009; **26**: 227-235 [PMID: 19584750 DOI: 10.1097/WNP.0b013e3181aed1a1]
  - 56 **Haas S**, Brock C, Krogh K, Gram M, Nissen TD, Lundby L, Laurberg S, Drewes AM. Cortical evoked potentials in response to rapid balloon distension of the rectum and anal canal. *Neurogastroenterol Motil* 2014; **26**: 862-873 [PMID: 24750321 DOI: 10.1111/nmo.12341]
  - 57 **Dejgaard A**, Gade A, Larsson H, Balle V, Parving A, Parving HH. Evidence for diabetic encephalopathy. *Diabet Med* 1991; **8**: 162-167 [PMID: 1827403]
  - 58 **Cooray GK**, Hyllienmark L, Brismar T. Decreased cortical connectivity and information flow in type 1 diabetes. *Clin Neurophysiol* 2011; **122**: 1943-1950 [PMID: 21474371 DOI: 10.1016/j.clinph.2011.03.007]
  - 59 **Cui D**, Liu J, Bian Z, Li Q, Wang L, Li X. Cortical source multivariate EEG synchronization analysis on amnesic mild cognitive impairment in type 2 diabetes. *ScientificWorldJournal* 2014; **2014**: 523216 [PMID: 25254248 DOI: 10.1155/2014/523216]
  - 60 **Fabris C**, Sparacino G, Sejling AS, Goljahani A, Duun-Henriksen J, Remvig LS, Juhl CB, Cobelli C. Hypoglycemia-related electroencephalogram changes assessed by multiscale entropy. *Diabetes Technol Ther* 2014; **16**: 688-694 [PMID: 24892361 DOI: 10.1089/dia.2013.0331]
  - 61 **Lelic D**, Brock C, Simrén M, Frøkjær JB, Søfteland E, Dimcevski G, Gregersen H, Drewes AM. The brain networks encoding visceral sensation in patients with gastrointestinal symptoms due to diabetic neuropathy. *Neurogastroenterol Motil* 2014; **26**: 46-58 [PMID: 24050116 DOI: 10.1111/nmo.12222]
  - 62 **Frøkjær JB**, Egsgaard LL, Graversen C, Søfteland E, Dimcevski G, Blauenfeldt RA, Drewes AM. Gastrointestinal symptoms in type-1 diabetes: is it all about brain plasticity? *Eur J Pain* 2011; **15**: 249-257 [PMID: 20813568 DOI: 10.1016/j.ejpain.2010.08.004]
  - 63 **Frøkjær JB**, Søfteland E, Graversen C, Dimcevski G, Egsgaard LL, Arendt-Nielsen L, Drewes AM. Central processing of gut pain in diabetic patients with gastrointestinal symptoms. *Diabetes Care* 2009; **32**: 1274-1277 [PMID: 19366960 DOI: 10.2337/dc09-0324]
  - 64 **Brock C**, Graversen C, Frøkjær JB, Søfteland E, Valeriani M, Drewes AM. Peripheral and central nervous contribution to gastrointestinal symptoms in diabetic patients with autonomic neuropathy. *Eur J Pain* 2013; **17**: 820-831 [PMID: 23239083 DOI: 10.1002/j.1532-2149.2012.00254.x]
  - 65 **Blatter DD**, Bigler ED, Gale SD, Johnson SC, Anderson CV, Burnett BM, Parker N, Kurth S, Horn SD. Quantitative volumetric analysis of brain MR: normative database spanning 5 decades of life. *ANR Am J Neuroradiol* 1995; **16**: 241-251 [PMID: 7726068]
  - 66 **Bigler ED**. Magnetic resonance imaging in the evaluation of cognitive function. *Pediatr Blood Cancer* 2014; **61**: 1724-1728 [PMID: 24920351 DOI: 10.1002/pbc.25110]
  - 67 **Buxton RB**. The physics of functional magnetic resonance imaging (fMRI). *Rep Prog Phys* 2013; **76**: 096601 [PMID: 24006360 DOI: 10.1088/0034-4885/76/9/096601]
  - 68 **Frøkjær JB**, Andersen LW, Brock C, Simrén M, Ljungberg M, Søfteland E, Dimcevski G, Yavarian Y, Gregersen H, Drewes AM. Altered brain microstructure assessed by diffusion tensor imaging in patients with diabetes and gastrointestinal symptoms. *Diabetes Care* 2013; **36**: 662-668 [PMID: 23139372 DOI: 10.2337/dc12-1131]
  - 69 **Moran C**, Phan TG, Chen J, Blizzard L, Beare R, Venn A, Münch G, Wood AG, Forbes J, Greenaway TM, Pearson S, Srikanth V. Brain atrophy in type 2 diabetes: regional distribution and influence on cognition. *Diabetes Care* 2013; **36**: 4036-4042 [PMID: 23939539 DOI: 10.2337/dc13-0143]
  - 70 **van Harten B**, de Leeuw FE, Weinstein HC, Scheltens P, Biessels GJ. Brain imaging in patients with diabetes: a systematic review. *Diabetes Care* 2006; **29**: 2539-2548 [PMID: 17065699 DOI: 10.2337/dc-06-1637]
  - 71 **Last D**, Alsop DC, Abduljalil AM, Marquis RP, de Bazelaire C, Hu K, Cavallerano J, Novak V. Global and regional effects of type 2 diabetes on brain tissue volumes and cerebral vasoreactivity. *Diabetes Care* 2007; **30**: 1193-1199 [PMID: 17290035 DOI: 10.2337/dc06-2052]
  - 72 **Jongen C**, van der Grond J, Kappelle LJ, Biessels GJ, Viergever MA, Pluim JP. Automated measurement of brain and white matter lesion volume in type 2 diabetes mellitus. *Diabetologia* 2007; **50**: 1509-1516 [PMID: 17492428 DOI: 10.1007/s00125-007-0688-y]
  - 73 **Roberts RO**, Knopman DS, Przybelski SA, Mielke MM, Kantarci K, Preboske GM, Senjem ML, Pankratz VS, Geda YE, Boeve BF, Ivnik RJ, Rocca WA, Petersen RC, Jack CR. Association of type 2 diabetes with brain atrophy and cognitive impairment. *Neurology* 2014; **82**: 1132-1141 [PMID: 24647028 DOI: 10.1212/wnl.0000000000000269]



- 74 **Kamiyama K**, Wada A, Sugihara M, Kurioka S, Hayashi K, Hayashi T, Yoshisako T, Yamamoto N, Tsuchie Y, Yamaguchi S, Sugimoto T, Kitagaki H. Potential hippocampal region atrophy in diabetes mellitus type 2: a voxel-based morphometry VSRAD study. *Jpn J Radiol* 2010; **28**: 266-272 [PMID: 20512543 DOI: 10.1007/s11604-009-0416-2]
- 75 **Frøkjær JB**, Brock C, Søfteland E, Dimcevski G, Gregersen H, Simrén M, M Drewes A. Macrostructural brain changes in patients with longstanding type 1 diabetes mellitus - a cortical thickness analysis study. *Exp Clin Endocrinol Diabetes* 2013; **121**: 354-360 [PMID: 23757052 DOI: 10.1055/s-0033-1345120]
- 76 **Bak AM**, Egeffjord L, Gejl M, Steffensen C, Stecher CW, Smidt K, Brock B, Rungby J. Targeting amyloid-beta by glucagon-like peptide -1 (GLP-1) in Alzheimer's disease and diabetes. *Expert Opin Ther Targets* 2011; **15**: 1153-1162 [PMID: 21749267 DOI: 10.1517/14728222.2011.600691]
- 77 **Holst JJ**, Gromada J. Role of incretin hormones in the regulation of insulin secretion in diabetic and nondiabetic humans. *Am J Physiol Endocrinol Metab* 2004; **287**: E199-E206 [PMID: 15271645 DOI: 10.1152/ajpendo.00545.2003]
- 78 **Orskov C**, Holst JJ, Nielsen OV. Effect of truncated glucagon-like peptide-1 [proglucagon-(78-107) amide] on endocrine secretion from pig pancreas, antrum, and nonantral stomach. *Endocrinology* 1988; **123**: 2009-2013 [PMID: 2901341 DOI: 10.1210/endo-123-4-2009]
- 79 **Holst JJ**, Burcelin R, Nathanson E. Neuroprotective properties of GLP-1: theoretical and practical applications. *Curr Med Res Opin* 2011; **27**: 547-558 [PMID: 21222567 DOI: 10.1185/03007995.2010.549466]
- 80 **Kazakos KA**, Sarafidis PA, Yovos JG. The impact of diabetic autonomic neuropathy on the incretin effect. *Med Sci Monit* 2008; **14**: CR213-CR220 [PMID: 18376350]
- 81 **Li X**, Pan W, Yang GZ, Di YN, Zhao F, Zhu LY, Jiang ZH. Proteome analysis of differential protein expression in brain of rats with type 1 diabetes mellitus. *Exp Clin Endocrinol Diabetes* 2011; **119**: 265-270 [PMID: 21472662 DOI: 10.1055/s-0031-1271705]
- 82 **Ramos-Rodríguez JJ**, Molina-Gil S, Ortiz-Barajas O, Jimenez-Palomares M, Perdomo G, Cozar-Castellano I, Lechuga-Sancho AM, Garcia-Alloza M. Central proliferation and neurogenesis is impaired in type 2 diabetes and prediabetes animal models. *PLoS One* 2014; **9**: e89229 [PMID: 24586614 DOI: 10.1371/journal.pone.0089229]
- 83 **Kodl CT**, Franc DT, Rao JP, Anderson FS, Thomas W, Mueller BA, Lim KO, Seaquist ER. Diffusion tensor imaging identifies deficits in white matter microstructure in subjects with type 1 diabetes that correlate with reduced neurocognitive function. *Diabetes* 2008; **57**: 3083-3089 [PMID: 18694971 DOI: 10.2337/db08-0724]
- 84 **Northam EA**, Rankins D, Lin A, Wellard RM, Pell GS, Finch SJ, Werther GA, Cameron FJ. Central nervous system function in youth with type 1 diabetes 12 years after disease onset. *Diabetes Care* 2009; **32**: 445-450 [PMID: 19151204 DOI: 10.2337/dc08-1657]

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## From bariatric to metabolic surgery: Looking for a “disease modifier” surgery for type 2 diabetes

Renzo Cordera, Gian Franco Adami

Renzo Cordera, Department of Internal Medicine, University of Genova, 16132 Genova, Italy

Gian Franco Adami, Department of Surgery, University of Genova, 16132 Genova, Italy

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**Correspondence to:** Renzo Cordera, MD, Professor of Endocrinology, Department of Internal Medicine, University of Genova, Viale Benedetto XV, 6, 16132 Genova, Italy. [record@unige.it](mailto:record@unige.it)  
Telephone: +39-10-3538947

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### Abstract

In this review the recent evolution of the comprehension of clinical and metabolic consequences of bariatric surgery is depicted. At the beginning bariatric surgery aim was a significant and durable weight loss. Later on,

it became evident that bariatric surgery was associated with metabolic changes, activated by unknown pathways, partially or totally independent of weight loss. Paradigm of this “metabolic” surgery is its effects on type 2 diabetes mellitus (T2DM). In morbid obese subjects it was observed a dramatic metabolic response leading to decrease blood glucose, till diabetes remission, before the achievement of clinically significant weight loss, opening the avenue to search for putative anti-diabetic “intestinal” factors. Both proximal duodenal (still unknown) and distal (GLP1) signals have been suggested as hormonal effectors of surgery on blood glucose decrease. Despite these findings T2DM remission was never considered a primary indication for bariatric surgery but only a secondary one. Recently T2DM remission in obese subjects with body mass index (BMI) greater than 35 has become a primary aim for surgery. This change supports the idea that “metabolic surgery” definition could more appropriate than bariatric, allowing to explore the possibility that metabolic surgery could represent a “disease modifier” for T2DM. Therefore, several patients have undergone surgery with a primary aim of a definitive cure of T2DM and today this surgery can be proposed as an alternative therapy. How much surgery can be considered truly metabolic is still unknown. To be truly “metabolic” it should be demonstrated that surgery could cause T2DM remission not only in subjects with BMI > 35 but also with BMI < 35 or even < 30. Available evidence on this topic is discussed in this mini-review.

**Key words:** Obesity; Severe obesity; Obesity related metabolic co-morbidities; Type 2 diabetes; Type 2 diabetes remission; Type 2 diabetes control; Bariatric/metabolic surgery

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**Core tip:** In severely obese patients with type 2 diabetes mellitus (T2DM), weight loss after bariatric surgery is often accompanied by long term remission of T2DM

and other obesity co-morbidities, these anti-diabetic outcomes are due both to body fat loss and to still unknown factors specifically regulated by surgery. On the other hand, the same surgical procedures in obese patients with a body mass index  $< 35$ , are associated with T2DM remission in only 50% of subjects. These findings raise the question whether bariatric surgery could be considered a metabolic one. We propose that "bariatric" surgery could be considered "metabolic" only in the case that it could demonstrate its effect on long lasting T2DM remission in morbid as well as in non-morbid obese subjects.

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## INTRODUCTION

Obesity and severe obesity was a condition well known already in the earlier ancient world: The famous rhetorician Claudius Aelian (170-235 A.D.) includes in his Historical Miscellany the careful report of a massively obese person, Dionysius tyrant of Heraclea, who being ashamed of his great body mass used to give audience to those who wished to confer with him by placing a chest in front of his body so that his whole body remained hidden except for his head<sup>[1]</sup>. However, substantially because of food unavailability, of extreme inequalities within population and of short mean life duration, the prevalence of obesity remained almost negligible until the XX century<sup>[2]</sup>. During the last 100 years in the Western world a greater availability of food for the great majority of the people, the prodigious improvements of preventive and clinical medicine and the reduction of social gap lead to a progressive increase of life expectancy with the consequent increased prevalence of obesity. In the Western developed countries in the 30's and 40's years of the last century to be slim and in shape became an ethic rather than a health condition: Overweight obese patients unable to loose weight were considered as lazy, ugly and stupid and become the object of a real social stigmatization<sup>[3,4]</sup>. After the Word War II, bariatric procedures were introduced in the United States as an effective anti-obesity surgical method, that allows significant weight loss also to patients who cannot reduce their usual food intake<sup>[5-7]</sup>. Obviously the social, stigmatization of obesity included both the obese patients seeking surgical treatment and the bariatric surgeons: The former are persons without will power, who threat their life for the whim to be slim, the latter are physicians taking care of a worthless issue<sup>[4]</sup>.

In the last decade this scenario changed radically. At the end of the XX century obesity has become an

increasing social problem in the western developed countries, with a vertiginous increase of prevalence and with great impact on health due severe co-morbidities. The positive trend of longevity obtained in the last years might be stopped and even reversed by the medical problems due to obesity: The baseline conditions being equal, an individual with body mass index (BMI) value over  $40 \text{ kg/m}^2$  has a 50% shorter life expectancy in comparison with a lean persons<sup>[8-10]</sup>. Taking into account these epidemiological findings, bariatric surgery is now accepted in the therapeutic armamentarium against obesity, and the surgical option is offered to severely (morbid) obese patients, that are now considered no more as lazy and stupid person but as complicated patients needing complex and tireless cures. Furthermore, the social and scientific consideration of bariatric surgeons, that have to cope with a very difficult task and to fight with the more prevalent chronic disease in the Western world, was sharply increased. At the beginning of XXI century bariatric surgery is regarded as a real branch of abdominal surgery.

The widespread use of bariatric procedures in different populations, by different surgical teams and in different socio-cultural contexts has clearly demonstrated that in the severely obese patients the surgically obtained weight loss is accompanied by the remission or by the improvement of most metabolic and cardiovascular co-morbidities of obesity, with a consequent increase in survival<sup>[11-14]</sup> from a research point of view the intriguing observation that most of metabolic effects are obtained well before a clinical meaningful weight loss has raised the hypothesis of a specific gastrointestinal effects of bariatric surgeries<sup>[15,16]</sup>. Therefore bariatric surgery community proposed to change the name of their discipline from "bariatric" to "metabolic" surgery, with the purpose of increasing their field of interest and of giving an ever greater deal of scientific background to their clinical practice.

By a pathophysiological point of view, in the obese patients the extra load of fat gives rise to the metabolic complications and to the cardiovascular disorders thus increasing morbidity and mortality: By determining a substantial weight loss and a marked reduction of body fat size, bariatric surgery acts on the metabolic complications only indirectly, and therefore a priori these procedures cannot be defined as true metabolic operations. In fact, in our opinion, a real metabolic procedure would influence the patient's metabolic status regardless of changes in body weight or in body fat size, or at least the body weight changes would play only a secondary role in the process.

Taking into account a clinical and scientific experience of more than thirty years, this brief review puts under close scrutiny the recent bariatric literature, with the aim to establish the possibility to define as "metabolic" the today bariatric surgery, specifically sleeve gastrectomy (SG), Roux-en-Y gastric bypass (RYGBP) and bilio-pancreatic diversion with its variations (BPD).

The placement of laparoscopic adjustable gastric

banding (LAGB) is the simplest and safest bariatric procedure. The operation causes a gastric restriction and a mechanical obstruction to transit of solid alimentary substrates: By consequence the usual food intake decrease with a resulting weight loss<sup>[17-19]</sup>. In the diabetic patients the reduction of food consumption and the drop of body mass size correspond to a normalization of blood glucose in 40%-50% of the cases, while postoperative improvement or resolution of dyslipidemia is only seldom observed<sup>[19-21]</sup>. However, in the patients having regained weight after the operation due to incongruous eating habits, the glycaemia increases again and diabetes relapses<sup>[21-24]</sup>. These data clearly indicate that the LAGB cannot be considered as a metabolic procedure and that the metabolic benefits experienced by some patients following the operation are essentially due to the weight loss.

In the last decade a great deal of studies have been carried out on the effects of the more complex bariatric procedures, such as the RYGBP, the SG and the BPD with their variations on the type 2 diabetes in the severely obese patients. In summary, published data indicate that in severe obese patients with type 2 diabetes the weight loss obtained following RYGBP is accompanied by diabetes remission in 60%-90% of patients and by an improvement of the diabetes in the remaining case, with a marked reduction of the need of anti-diabetic therapy: These positive results are maintained at 5 and 10 years after the operation<sup>[25-30]</sup>. Furthermore, in comparison with non operated severely obese patients with T2DM, the individuals submitted to RYGBP increase their longevity and experience a reduction in the rate of cardiovascular events<sup>[14]</sup>.

T2DM remission rate is higher after BPD in comparison with the other types of procedures<sup>[26-29]</sup>, the studies carried out in large cohort of subjects and in different institutions showing a stable and long lasting (10 years) T2DM remission in more than the 86% of patients. The long term positive metabolic outcome RYGBP and SL are similar and nearly occurs in 60% of the cases, though long term results following SG are not yet fully available<sup>[31,32]</sup>. Obviously the remission rate after RYGBP and BPD depends on preoperative variables: In the obese patients with a longer pre-operative T2DM duration and in those who were in insulin therapy before the operation the remission rate was about 70% and 50%, respectively, with a marked metabolic improvement in the others<sup>[33-35]</sup>.

Following RYGB and BPD blood glucose control is usually observed early, when BMI values are still in the morbid obesity range, with a rapid fall of serum glucose, insulin and insulin sensitivity values towards physiological range<sup>[15,16]</sup>. The new functional - anatomic conditions of the upper gastrointestinal tract due to the operation might causes a change of intestinal hormone secretion patterns, that would be responsible of the metabolic changes observed. The bypass of proximal intestine from the food transit and the early contact of non-digested foods with more distal intestinal

loops increase the secretion of GIP and GLP-1 that have gastrointestinal and insulin-tropic effects<sup>[36-38]</sup>. Furthermore, the passage of partially digested aliments through distal ileum cause an increase of secretion in PYY, a distal entero-hormone that specifically stimulates satiety<sup>[39]</sup>. For BPD, the fat intestinal malabsorption due to the operation causes a significant lipid deprivation from the first postoperative days and then a marked decrease of the intracellular lipid storage, that sharply increases muscle insulin sensitivity<sup>[40,41]</sup>. After SL similar changes of gastric and intestinal hormones were observed in spite of the lack of duodenal exclusion<sup>[42]</sup>. In summary, several studies have demonstrated in both diabetic and non diabetic severely obese patient a metabolic recovery early after the operation, when body weight is still in the obese range. Furthermore, as it results by the clinical experience of many bariatric surgery teams and as it is indicated by a great deal of literature data, the post obese individuals operated of BPD and RYGBP achieve a BMI of stabilization in the overweight range (28-30 kg/m<sup>2</sup>), that is maintained indefinitely<sup>[26,43-45]</sup>. In spite of an higher than normal body weight, in the a cohort of post obese persons submitted to RYGB or BPD the occurrence of a new case of diabetes is highly unusual. In addition, when the severely obese T2DM patients with positive and negative post BPD and post RYGB metabolic outcome were compared, a closely similar weight loss was observed<sup>[34,35]</sup>, suggesting that diabetes remission could be due not exclusively to surgery but to a combination of surgery with the right subject. These facts clearly evidence the lack of relationship between the weight status and the presence of diabetes after bariatric surgery, and indicate that in these cases the operations have to be considered as true metabolic operation. In the severely obese patients with T2DM submitted to BPD, glucose, glycated hemoglobin serum concentration and insulin sensitivity are normalized for very long time. Also insulin secretion improves as indicated by post surgery recovery of early insulin secretion stimulated by *iv* glucose. The most striking result of surgery is the reversal of the progressive failure of beta cell, as observed instead in non operated T2DM, due to metabolic noxa<sup>[46]</sup>. For this reason, in severely obese patients BPD could be considered as a true diabetes modifier therapy<sup>[15,21,47]</sup>.

As far as lipid metabolism is concerned, in pre-operatively patients with atherogenic dyslipidemia a reduction of serum triglycerides and total cholesterol is usually observed after BPD and RYGBP, and values were satisfactory maintained at long term following operation<sup>[25,44,45,48]</sup>. After RYGBP the improvement of lipid pattern is substantially due to the reduction of food intake and to qualitative changes in alimentary substrates. On the contrary, after BPD the lipid malabsorption leads to a decrease of distal gut uptake of bile salts, thus obviously determining a size reduction of body cholesterol pool<sup>[45,49]</sup>. HDL cholesterol concentration is generally unaffected by bariatric surgery and by the



massive weight loss; but a significant increase of serum HDL cholesterol value was observed at long and at very long term following the bariatric operation, most likely for decreased insulin resistance and lifestyle changes with progressively increases its physical mobility<sup>[45]</sup>. Therefore, regarding total cholesterol, BPD acts as a metabolic operation, while the effects of bariatric surgery on the other components of lipid metabolism are substantially accounted for by the weight loss and by the changes in lifestyle and food consumption. The above reported clinical and experimental results are suggestive for the possibility to change the primary aim of bariatric surgery from weight loss to T2DM remission.

In clinical practice, the majority of T2DM patients are not morbidly obese, their BMI typically ranging between 25 and 35 kg/m<sup>2</sup>. By the other hand, as said above, BPD and RYGBP act on severely obese patients with a real metabolic mechanism, and T2DM, as well as other comorbidities such as hypertension and dyslipidemia, improve at an early time point after surgery, independent of major weight loss<sup>[15,16,37,38,41]</sup>. Recently, both observational<sup>[50-55]</sup> and randomized controlled trials<sup>[34,56-61]</sup> have indicated that metabolic surgery in severely obese patients might be superior to medical therapy with regard to T2DM remission and glycemic control. Consequently, bariatric-metabolic surgery has been proposed as a treatment option for T2DM also in patients with overweight or obesity of mild degree, because it potentially leads to less morbidity and mortality in the long-term. Furthermore, an adjustment of treatment guidelines favoring metabolic surgery in non severely obese patients would have a major impact on global health care. Recently a very accurate metanalysis and review studies were carried out, that clearly showed the short-term superiority of metabolic surgery to medical treatment, with regard to T2DM remission, glycemic control, and remission of associated comorbidities<sup>[62-64]</sup>. However, a relevant heterogeneity of data according to the type of surgical procedure employed and to the characteristics of the patients was observed. In comparison with standard anti-diabetic therapy, in patients submitted to the LAGB or SG the metabolic outcome was better. However, the attainment of normal serum glucose concentration was observed in less than 30% of operated Patients, the results being substantially due to the weight loss and tending to disappear with weight regain<sup>[55,56,61]</sup>. The metabolic effect of the more complex procedures was higher, the procedures leading to a fasting blood glucose control or normalization in 50%-60% of the cases within one years after the operation. Therefore both RYGBP and BPD might act as metabolic operations also in T2DM individuals with simple overweight or obesity of mild degree; however the overall results were far less striking and evident than those usually observed in severely obese patients. The rate of postoperative patients with diabetes remission one year after BPD and RYGBP, thought in any case greater than that observed in patients undergoing medical therapy, was consistently

lower in comparison to the rate of postoperative diabetes remission of T2DM in morbidly obese patients. Since the nature of the surgical procedure is the same, the discordant results are most likely due to the diabetes characteristics and/or to the patient's obesity degree. Furthermore, as far as the postoperative diabetes remission in the non morbid obese patients, at the present time only data at short term are available. As said above, type 2 diabetes is a progressive disease, for both a gradual increase in insulin resistance and decrease of beta cell mass due to genetic defects and to glyco-lipotoxicity<sup>[46]</sup>. In morbid obese patients, bariatric surgery modifies diabetes natural history by steadily restoring insulin sensitivity of beta cell secretion, evidenced even at five years following surgery<sup>[47]</sup>.

In T2DM without morbid obesity the picture is still unclear and only the long term functional and clinical results will establish whether RYGBP and BPD act as metabolic operation. An improved and durable rate of diabetes remission could demonstrate that bariatric operation resulted in stopping or reverting the natural history of diabetes, in this demonstrating a metabolic action. On the contrary, if the positive metabolic outcome observed after one year will disappear, it would be demonstrated that the progressive detrimental trend of T2DM is not directly influenced by the operation, and that the metabolic benefits observed at the first postoperative year are substantially accounted for weight loss and by the reduction of insulin resistance, in that excluding a direct metabolic effect of the operation. At one year after BPD, in T2DM overweight-mildly obese patients, the diabetes remission and glycemic control was observed in 40% and 70% of the cases, respectively, while the acute insulin response was not completely restored, differently from what observed in T2DM morbidly obese patients<sup>[47,65-67]</sup>. This already suggests that the positive outcome is substantially accounted for by weight loss and by insulin resistance decrease and that BPD has only a little metabolic effect. Longer term studies will clarify this issue.

At the present time, for T2DM patient, bariatric surgery can change to a metabolic one depending on the clinical characteristic of the patient itself. In other words it is possible, even if not completely proofed that the more obese patient before surgery the more metabolically effective is BPD, independently, at least partially, by weight loss. This consideration is based on the assumption that the amount of beta cells is increased in morbid obese subjects, even if already diabetic: recovery from insulin resistance due mostly to a greatly reduction of caloric intake can cure T2DM, because of an enough amount of circulating available insulin.

In summary, this review suggests that the term metabolic surgery should not to be employed as a synonymous of bariatric surgery. Gastric restrictive surgery gives positive metabolic outcomes only in a minority of the patients, and the results are substantially accounted for by the weight loss and are poorly maintained at long term. In the T2DM severely obese patients

RYGBP and BPD obtain astonishing metabolic outcomes independently of weight loss. In this subset of subjects, in spite of the progressive detrimental characteristics of T2DM, the results are maintained throughout the years, so that the operation could be regarded as a true diabetes modifier therapy. In morbidly obese patients BPD and RYGBP actually are metabolic operations. This could not be the case in T2DM patients with simply overweight or obesity of mid degree: However, this lack of metabolic efficacy is not dependent on the operation itself but on the characteristics of the patients.

## REFERENCES

- 1 **Bevegni C**, Adami GF. Obesity and obesity surgery in ancient Greece. *Obes Surg* 2003; **13**: 808-809 [PMID: 14627484]
- 2 **Haslam D**. Obesity: a medical history. *Obes Rev* 2007; **8** Suppl 1: 31-36 [PMID: 17316298]
- 3 **Eknoyan G**. A history of obesity, or how what was good became ugly and then bad. *Adv Chronic Kidney Dis* 2006; **13**: 421-427 [PMID: 17045228]
- 4 **Puhl RM**, Heuer CA. The stigma of obesity: a review and update. *Obesity* (Silver Spring) 2009; **17**: 941-964 [PMID: 19165161]
- 5 **Mason EE**, Ito C. Gastric bypass. *Ann Surg* 1969; **170**: 329-339 [PMID: 5804373]
- 6 **Scopinaro N**, Gianetta E, Civalleri D. Small bowel bypass for obesity. *Ann Surg* 1977; **186**: 776-777 [PMID: 603283]
- 7 **Kirby RM**, Ismail T, Crowson M, Baddeley RM. Gastric banding in the treatment of morbid obesity. *Br J Surg* 1989; **76**: 490-492 [PMID: 2736363]
- 8 **Kushner RF**. Body weight and mortality. *Nutr Rev* 1993; **51**: 127-136 [PMID: 8332284]
- 9 **Berg C**, Rosengren A, Aires N, Lappas G, Torén K, Thelle D, Lissner L. Trends in overweight and obesity from 1985 to 2002 in Göteborg, West Sweden. *Int J Obes* (Lond) 2005; **29**: 916-924 [PMID: 15852045]
- 10 **Gregg EW**, Cheng YJ, Cadwell BL, Imperatore G, Williams DE, Flegal KM, Narayan KM, Williamson DF. Secular trends in cardiovascular disease risk factors according to body mass index in US adults. *JAMA* 2005; **293**: 1868-1874 [PMID: 15840861]
- 11 **MacDonald KG**, Long SD, Swanson MS, Brown BM, Morris P, Dohm GL, Pories WJ. The gastric bypass operation reduces the progression and mortality of non-insulin-dependent diabetes mellitus. *J Gastrointest Surg* 1997; **1**: 213-20; discussion 220 [PMID: 9834350]
- 12 **Adams TD**, Gress RE, Smith SC, Halverson RC, Simper SC, Rosamond WD, Lamonte MJ, Stroup AM, Hunt SC. Long-term mortality after gastric bypass surgery. *N Engl J Med* 2007; **357**: 753-761 [PMID: 17715409]
- 13 **Sjöström L**, Narbro K, Sjöström CD, Karason K, Larsson B, Wedel H, Lystig T, Sullivan M, Bouchard C, Carlsson B, Bengtsson C, Dahlgren S, Gummesson A, Jacobson P, Karlsson J, Lindroos AK, Lönroth H, Näslund I, Olbers T, Stenlöf K, Torgerson J, Agren G, Carlsson LM. Effects of bariatric surgery on mortality in Swedish obese subjects. *N Engl J Med* 2007; **357**: 741-752 [PMID: 17715408]
- 14 **Sjöström L**, Peltonen M, Jacobson P, Ahlin S, Andersson-Assarsson J, Anveden Å, Bouchard C, Carlsson B, Karason K, Lönroth H, Näslund I, Sjöström E, Taube M, Wedel H, Svensson PA, Sjöholm K, Carlsson LM. Association of bariatric surgery with long-term remission of type 2 diabetes and with microvascular and macrovascular complications. *JAMA* 2014; **311**: 2297-2304 [PMID: 24915261 DOI: 10.1001/jama.2014.5988]
- 15 **Guidone C**, Manco M, Valera-Mora E, Iaconelli A, Gniuli D, Mari A, Nanni G, Castagneto M, Calvani M, Mingrone G. Mechanisms of recovery from type 2 diabetes after malabsorptive bariatric surgery. *Diabetes* 2006; **55**: 2025-2031 [PMID: 16804072]
- 16 **Adami GF**, Cordera R, Camerini G, Marinari GM, Scopinaro N. Recovery of insulin sensitivity in obese patients at short term after biliopancreatic diversion. *J Surg Res* 2003; **113**: 217-221 [PMID: 12957132]
- 17 **O'Brien PE**, MacDonald L, Anderson M, Brennan L, Brown WA. Long-term outcomes after bariatric surgery: fifteen-year follow-up of adjustable gastric banding and a systematic review of the bariatric surgical literature. *Ann Surg* 2013; **257**: 87-94 [PMID: 23235396 DOI: 10.1097/SLA.0b013e31827b6c02]
- 18 **Sultan S**, Gupta D, Parikh M, Youn H, Kurian M, Fielding G, Ren-Fielding C. Five-year outcomes of patients with type 2 diabetes who underwent laparoscopic adjustable gastric banding. *Surg Obes Relat Dis* 2010; **6**: 373-376 [PMID: 20627708 DOI: 10.1016/j.soard.2010.02.043]
- 19 **Favretti F**, Segato G, Ashton D, Busetto L, De Luca M, Mazza M, Ceoloni A, Banzato O, Calo E, Enzi G. Laparoscopic adjustable gastric banding in 1,791 consecutive obese patients: 12-year results. *Obes Surg* 2007; **17**: 168-175 [PMID: 17476867]
- 20 **Dixon JB**, Murphy DK, Segel JE, Finkelstein EA. Impact of laparoscopic adjustable gastric banding on type 2 diabetes. *Obes Rev* 2012; **13**: 57-67 [PMID: 21880108 DOI: 10.1111/j.1467-789X.2011.00928]
- 21 **Buchwald H**, Estok R, Fahrenbach K, Banel D, Jensen MD, Pories WJ, Bantle JP, Sledge I. Weight and type 2 diabetes after bariatric surgery: systematic review and meta-analysis. *Am J Med* 2009; **122**: 248-256.e5 [PMID: 19272486 DOI: 10.1016/j.amjmed.2008.09.041]
- 22 **Levy P**, Fried M, Santini F, Finer N. The comparative effects of bariatric surgery on weight and type 2 diabetes. *Obes Surg* 2007; **17**: 1248-1256 [PMID: 18074502]
- 23 **Merlotti C**, Morabito A, Pontiroli AE. Prevention of type 2 diabetes: a systematic review and meta-analysis of different intervention strategies. *Diabetes Obes Metab* 2014; **16**: 719-727 [PMID: 24476122 DOI: 10.1111/dom.12270]
- 24 **Kasza J**, Brody F, Vaziri K, Scheffey C, McMullan S, Wallace B, Khambaty F. Analysis of poor outcomes after laparoscopic adjustable gastric banding. *Surg Endosc* 2011; **25**: 41-47 [PMID: 20589514 DOI: 10.1007/s00464-010-1126-7]
- 25 **Scopinaro N**, Marinari GM, Camerini GB, Papadia FS, Adami GF. Specific effects of biliopancreatic diversion on the major components of metabolic syndrome: a long-term follow-up study. *Diabetes Care* 2005; **28**: 2406-2411 [PMID: 16186271]
- 26 **Colquitt JL**, Pickett K, Loveman E, Frampton GK. Surgery for weight loss in adults. *Cochrane Database Syst Rev* 2014; **8**: CD003641 [PMID: 25105982 DOI: 10.1002/14651858]
- 27 **Ribaric G**, Buchwald JN, McGlennon TW. Diabetes and weight in comparative studies of bariatric surgery vs conventional medical therapy: a systematic review and meta-analysis. *Obes Surg* 2014; **24**: 437-455 [PMID: 24374842 DOI: 10.1007/s11695-013-1160-3]
- 28 **Gloy VL**, Briel M, Bhatt DL, Kashyap SR, Schauer PR, Mingrone G, Bucher HC, Nordmann AJ. Bariatric surgery versus non-surgical treatment for obesity: a systematic review and meta-analysis of randomised controlled trials. *BMJ* 2013; **347**: f5934 [PMID: 24149519 DOI: 10.1136/bmj.f5934]
- 29 **Guo X**, Liu X, Wang M, Wei F, Zhang Y, Zhang Y. The effects of bariatric procedures versus medical therapy for obese patients with type 2 diabetes: meta-analysis of randomized controlled trials. *Biomed Res Int* 2013; **2013**: 410609 [PMID: 23971035 DOI: 10.1155/2013/410609]
- 30 **Heneghan HM**, Nissen S, Schauer PR. Gastrointestinal surgery for obesity and diabetes: weight loss and control of hyperglycemia. *Curr Atheroscler Rep* 2012; **14**: 579-587 [PMID: 23054661 DOI: 10.1007/s11883-012-0285-5]
- 31 **Wang MC**, Guo XH, Zhang YW, Zhang YL, Zhang HH, Zhang YC. Laparoscopic Roux-en-Y gastric bypass versus sleeve gastrectomy for obese patients with Type 2 diabetes: a meta-analysis of randomized controlled trials. *Am Surg* 2015; **81**: 166-171 [PMID: 25642879]
- 32 **Yip S**, Plank LD, Murphy R. Gastric bypass and sleeve gastrectomy for type 2 diabetes: a systematic review and meta-analysis of outcomes. *Obes Surg* 2013; **23**: 1994-2003 [PMID: 23955521 DOI: 10.1007/s11695-013-1030-z]

- 33 **Khanna V**, Malin SK, Bena J, Abood B, Pothier CE, Bhatt DL, Nissen S, Watanabe R, Brethauer SA, Schauer PR, Kirwan JP, Kashyap SR. Adults with long-duration type 2 diabetes have blunted glycemic and  $\beta$ -cell function improvements after bariatric surgery. *Obesity* (Silver Spring) 2015; **23**: 523-526 [PMID: 25651277 DOI: 10.1002/oby.21021]
- 34 **Schauer PR**, Kashyap SR, Wolski K, Brethauer SA, Kirwan JP, Pothier CE, Thomas S, Abood B, Nissen SE, Bhatt DL. Bariatric surgery versus intensive medical therapy in obese patients with diabetes. *N Engl J Med* 2012; **366**: 1567-1576 [PMID: 22449319 DOI: 10.1056/NEJMoa1200225]
- 35 **Scopinaro N**, Camerini G, Papadia F, Catalano MF, Carlini F, Adami GF. In diabetes obese patients the impact of biliopancreatic diversion on glycemic control for the main predicting factors is maintained at long term. *SOARD* 2015; In press
- 36 **Rubino F**, R'bib SL, del Genio F, Mazumdar M, McGraw TE. Metabolic surgery: the role of the gastrointestinal tract in diabetes mellitus. *Nat Rev Endocrinol* 2010; **6**: 102-109 [PMID: 20098450 DOI: 10.1038/nrendo.2009.268]
- 37 **Castagneto-Gissey L**, Mingrone G. Insulin sensitivity and secretion modifications after bariatric surgery. *J Endocrinol Invest* 2012; **35**: 692-698 [PMID: 22732257 DOI: 10.3275/8470]
- 38 **Mingrone G**, Castagneto-Gissey L. Mechanisms of early improvement/resolution of type 2 diabetes after bariatric surgery. *Diabetes Metab* 2009; **35**: 518-523 [PMID: 20152737 DOI: 10.1016/S1262-3636(09)73459-7]
- 39 **Ionut V**, Burch M, Youdim A, Bergman RN. Gastrointestinal hormones and bariatric surgery-induced weight loss. *Obesity* (Silver Spring) 2013; **21**: 1093-1103 [PMID: 23512841 DOI: 10.1002/oby.20364]
- 40 **Greco AV**, Mingrone G, Giancaterini A, Manco M, Morroni M, Cinti S, Granzotto M, Vettor R, Camastra S, Ferrannini E. Insulin resistance in morbid obesity: reversal with intramyocellular fat depletion. *Diabetes* 2002; **51**: 144-151 [PMID: 11756334]
- 41 **Adami GF**, Parodi RC, Papadia F, Marinari G, Camerini G, Corvisieri R, Scopinaro N. Magnetic resonance spectroscopy facilitates assessment of intramyocellular lipid changes: a preliminary short-term study following biliopancreatic diversion. *Obes Surg* 2005; **15**: 1233-1237 [PMID: 16259877]
- 42 **Nannipieri M**, Baldi S, Mari A, Colligiani D, Guarino D, Camastra S, Barsotti E, Berta R, Moriconi D, Bellini R, Anselmino M, Ferrannini E. Roux-en-Y gastric bypass and sleeve gastrectomy: mechanisms of diabetes remission and role of gut hormones. *J Clin Endocrinol Metab* 2013; **98**: 4391-4399 [PMID: 24057293 DOI: 10.1210/jc.2013-2538]
- 43 **Papamargaritis D**, le Roux CW, Sioka E, Koukoulis G, Tzovaras G, Zacharoulis D. Changes in gut hormone profile and glucose homeostasis after laparoscopic sleeve gastrectomy. *Surg Obes Relat Dis* 2013; **9**: 192-201 [PMID: 23183113 DOI: 10.1016/j.soard.2012.08.007]
- 44 **Puzziferri N**, Roshek TB, Mayo HG, Gallagher R, Belle SH, Livingston EH. Long-term follow-up after bariatric surgery: a systematic review. *JAMA* 2014; **312**: 934-942 [PMID: 25182102 DOI: 10.1001/jama.2014.10706]
- 45 **Scopinaro N**, Adami GF, Marinari GM, Gianetta E, Traverso E, Friedman D, Camerini G, Baschieri G, Simonelli A. Biliopancreatic diversion. *World J Surg* 1998; **22**: 936-946 [PMID: 9717419]
- 46 **Halban PA**, Polonsky KS, Bowden DW, Hawkins MA, Ling C, Mather KJ, Powers AC, Rhodes CJ, Sussel L, Weir GC.  $\beta$ -cell failure in type 2 diabetes: postulated mechanisms and prospects for prevention and treatment. *Diabetes Care* 2014; **37**: 1751-1758 [PMID: 24812433 DOI: 10.2337/dc14-0396]
- 47 **Scopinaro N**, Camerini G, Papadia F, Catalano MF, Andraghetti G, Cordera R, Adami GF. Long term clinical and functional impact of biliopancreatic diversion on type 2 diabetes in morbidly and not morbidly obese patients. *Diabetes Care* 2015; In press
- 48 **Søvik TT**, Aasheim ET, Taha O, Engström M, Fagerland MW, Björkman S, Kristinsson J, Birkeland KI, Mala T, Olbers T. Weight loss, cardiovascular risk factors, and quality of life after gastric bypass and duodenal switch: a randomized trial. *Ann Intern Med* 2011; **155**: 281-291 [PMID: 21893621 DOI: 10.7326/0003-4819-155-5-201109060-00005]
- 49 **Benetti A**, Del Puppo M, Crosignani A, Veronelli A, Masci E, Frigè F, Micheletto G, Panizzo V, Pontiroli AE. Cholesterol metabolism after bariatric surgery in grade 3 obesity: differences between malabsorptive and restrictive procedures. *Diabetes Care* 2013; **36**: 1443-1447 [PMID: 23275360 DOI: 10.2337/dc12-1737]
- 50 **Chiellini C**, Rubino F, Castagneto M, Nanni G, Mingrone G. The effect of bilio-pancreatic diversion on type 2 diabetes in patients with BMI < 35 kg/m<sup>2</sup>. *Diabetologia* 2009; **52**: 1027-1030 [PMID: 19308351 DOI: 10.1007/s00125-009-1333-8]
- 51 **Scopinaro N**, Adami GF, Papadia FS, Camerini G, Carlini F, Fried M, Briatore L, D'Alessandro G, Andraghetti G, Cordera R. Effects of biliopancreatic diversion on type 2 diabetes in patients with BMI 25 to 35. *Ann Surg* 2011; **253**: 699-703 [PMID: 21475009 DOI: 10.1097/SLA.0b013e318203ae44]
- 52 **Serrot FJ**, Dorman RB, Miller CJ, Slusarek B, Sampson B, Sick BT, Leslie DB, Buchwald H, Ikramuddin S. Comparative effectiveness of bariatric surgery and nonsurgical therapy in adults with type 2 diabetes mellitus and body mass index < 35 kg/m<sup>2</sup>. *Surgery* 2011; **150**: 684-691 [PMID: 22000180 DOI: 10.1016/j.surg.2011.07.069]
- 53 **Abbatini F**, Capoccia D, Casella G, Coccia F, Leonetti F, Basso N. Type 2 diabetes in obese patients with body mass index of 30-35 kg/m<sup>2</sup>: sleeve gastrectomy versus medical treatment. *Surg Obes Relat Dis* 2012; **8**: 20-24 [PMID: 21924686 DOI: 10.1016/j.soard.2011.06.015]
- 54 **Geloneze B**, Geloneze SR, Chaim E, Hirsch FF, Felici AC, Lambert G, Tambascia MA, Pareja JC. Metabolic surgery for non-obese type 2 diabetes: incretins, adipocytokines, and insulin secretion/resistance changes in a 1-year interventional clinical controlled study. *Ann Surg* 2012; **256**: 72-78 [PMID: 22664560]
- 55 **Scopinaro N**, Adami GF, Papadia FS, Camerini G, Carlini F, Briatore L, Andraghetti G, Catalano M, Cordera R. Effects of gastric bypass on type 2 diabetes in patients with BMI 30 to 35. *Obes Surg* 2014; **24**: 1036-1043 [PMID: 24647849 DOI: 10.1007/s11695-014-1206-1]
- 56 **Dixon JB**, O'Brien PE, Playfair J, Chapman L, Schachter LM, Skinner S, Proietto J, Bailey M, Anderson M. Adjustable gastric banding and conventional therapy for type 2 diabetes: a randomized controlled trial. *JAMA* 2008; **299**: 316-323 [PMID: 18212316 DOI: 10.1001/jama.299.3.316]
- 57 **Ikramuddin S**, Korner J, Lee WJ, Connett JE, Inabnet WB, Billington CJ, Thomas AJ, Leslie DB, Chong K, Jeffery RW, Ahmed L, Vella A, Chuang LM, Bessler M, Sarr MG, Swain JM, Laqua P, Jensen MD, Bantle JP. Roux-en-Y gastric bypass vs intensive medical management for the control of type 2 diabetes, hypertension, and hyperlipidemia: the Diabetes Surgery Study randomized clinical trial. *JAMA* 2013; **309**: 2240-2249 [PMID: 23736733 DOI: 10.1001/jama.2013.5835]
- 58 **Liang Z**, Wu Q, Chen B, Yu P, Zhao H, Ouyang X. Effect of laparoscopic Roux-en-Y gastric bypass surgery on type 2 diabetes mellitus with hypertension: a randomized controlled trial. *Diabetes Res Clin Pract* 2013; **101**: 50-56 [PMID: 23706413 DOI: 10.1016/j.diabres.2013.04.005]
- 59 **Courcoulas AP**, Goodpaster BH, Eagleton JK, Belle SH, Kalarchian MA, Lang W, Toledo FG, Jakicic JM. Surgical vs medical treatments for type 2 diabetes mellitus: a randomized clinical trial. *JAMA Surg* 2014; **149**: 707-715 [PMID: 24899268 DOI: 10.1001/jamasurg.2014.467]
- 60 **Halperin F**, Ding SA, Simonson DC, Panosian J, Goebel-Fabbri A, Wewalka M, Hamdy O, Abrahamson M, Clancy K, Foster K, Lautz D, Vernon A, Goldfine AB. Roux-en-Y gastric bypass surgery or lifestyle with intensive medical management in patients with type 2 diabetes: feasibility and 1-year results of a randomized clinical trial. *JAMA Surg* 2014; **149**: 716-726 [PMID: 24899464 DOI: 10.1001/jamasurg.2014.514]
- 61 **Wentworth JM**, Playfair J, Laurie C, Ritchie ME, Brown WA, Burton P, Shaw JE, O'Brien PE. Multidisciplinary diabetes care with and without bariatric surgery in overweight people: a randomised

- controlled trial. *Lancet Diabetes Endocrinol* 2014; **2**: 545-552 [PMID: 24731535 DOI: 10.1016/S2213-8587(14)70066-X]
- 62 **Fried M**, Ribaric G, Buchwald JN, Svacina S, Dolezalova K, Scopinaro N. Metabolic surgery for the treatment of type 2 diabetes in patients with BMI  $\leq 35$  kg/m<sup>2</sup>: an integrative review of early studies. *Obes Surg* 2010; **20**: 776-790 [PMID: 20333558 DOI: 10.1007/s11695-010-0113-3]
- 63 **Müller-Stich BP**, Senft JD, Warschkow R, Kenngott HG, Billeter AT, Vit G, Helfert S, Diener MK, Fischer L, Büchler MW, Nawroth PP. Surgical versus medical treatment of type 2 diabetes mellitus in nonseverely obese patients: a systematic review and meta-analysis. *Ann Surg* 2015; **261**: 421-429 [PMID: 25405560 DOI: 10.1097/SLA.0000000000001014]
- 64 **Li Q**, Chen L, Yang Z, Ye Z, Huang Y, He M, Zhang S, Feng X, Gong W, Zhang Z, Zhao W, Liu C, Qu S, Hu R. Metabolic effects of bariatric surgery in type 2 diabetic patients with body mass index  $\leq 35$  kg/m<sup>2</sup>. *Diabetes Obes Metab* 2012; **14**: 262-270 [PMID: 22051116 DOI: 10.1111/j.1463-1326.2011.01524]
- 65 **Scopinaro N**, Adami GF, Papadia FS, Camerini G, Carlini F, Briatore L, D'Alessandro G, Parodi C, Weiss A, Andraghetti G, Catalano M, Cordera R. The effects of biliopancreatic diversion on type 2 diabetes mellitus in patients with mild obesity (BMI 30-35 kg/m<sup>2</sup>) and simple overweight (BMI 25-30 kg/m<sup>2</sup>): a prospective controlled study. *Obes Surg* 2011; **21**: 880-888 [PMID: 21541815 DOI: 10.1007/s11695-011-0407-0]
- 66 **Briatore L**, Salani B, Andraghetti G, Maggi D, Adami GF, Scopinaro N, Cordera R. Beta-cell function improvement after biliopancreatic diversion in subjects with type 2 diabetes and morbid obesity. *Obesity* (Silver Spring) 2010; **18**: 932-936 [PMID: 20186136 DOI: 10.1038/oby.2010.28]
- 67 **Polyzogopoulou EV**, Kalfarentzos F, Vagenakis AG, Alexandrides TK. Restoration of euglycemia and normal acute insulin response to glucose in obese subjects with type 2 diabetes following bariatric surgery. *Diabetes* 2003; **52**: 1098-1103 [PMID: 12716738]

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Room 903, Building D, Ocean International Center,  
No. 62 Dongsihuan Zhonglu, Chaoyang District,  
Beijing 100025, China  
Telephone: +86-10-85381891  
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## Diabetes in migrants and ethnic minorities in a changing World

Luca Montesi, Maria Turchese Caletti, Giulio Marchesini

Luca Montesi, Maria Turchese Caletti, Giulio Marchesini,  
 Department of Medical and Surgical Sciences, S.Orsola-Malpighi  
 Hospital, "Alma Mater Studiorum" University, I-40138 Bologna,  
 Italy

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**Correspondence to:** Giulio Marchesini, MD, PhD, Department of Medical and Surgical Sciences, S.Orsola-Malpighi Hospital, "Alma Mater Studiorum" University, Via Massarenti n°9, I-40138 Bologna, Italy. [giulio.marchesini@unibo.it](mailto:giulio.marchesini@unibo.it)  
 Telephone: +39-051-2144889  
 Fax: +39-051-6364502

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### Abstract

On a worldwide scale, the total number of migrants exceeds 200 million and is not expected to reduce, fuelled by the economic crisis, terrorism and wars, generating increasing clinical and administrative problems to National Health Systems. Chronic non-communicable diseases (NCD), and specifically diabetes, are on the front-line, due to the high number of cases at risk, duration and cost of diseases, and availability of effective measures of prevention and treatment. We reviewed the documents of International Agencies on migration and performed a PubMed search of existing literature, focusing on the differences in the prevalence of diabetes between migrants and native people, the prevalence of NCD in migrants *vs* rates in the countries of origin, diabetes convergence, risk of diabetes progression and standard of care in migrants. Even in universalistic healthcare systems, differences in socioeconomic status and barriers generated by the present culture of biomedicine make high-risk ethnic minorities under-treated and not protected against inequalities. Underutilization of drugs and primary care services in specific ethnic groups are far from being money-saving, and might produce higher hospitalization rates due to disease progression and complications. Efforts should be made to favor screening and treatment programs, to adapt education programs to specific cultures, and to develop community partnerships.

**Key words:** Migrants; Ethnic minorities; Diabetes; Health Systems; Non communicable diseases; Genetics; Socioeconomic development; Social determinants

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**Core tip:** At global level the phenomenon of migration of people is not expected to slow down in the next years, generating a multitude of clinical problems and economic

cost for the National Health System. The increasing burden of chronic diseases, particularly diabetes, in migrant minority populations is today a major public health challenge for several countries, mainly in Europe, fuelled by the economic crisis, inequalities, terrorism and wars. Even in a universalistic healthcare system, differences in socioeconomic status and barriers generated by the present culture of biomedicine might make high-risk ethnic minorities under-treated and not protected against inequalities. Our objective is to pinpoint the problems arising in the prevention and treatment of diabetes on a worldwide scale, aiming to give support to healthcare systems in the provision of effective interventions.

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## INTRODUCTION

### **Migration, migrants and Health Systems**

Migration is an ancient phenomenon, dating back to the time of our African ancestors and occurring on a variety of levels (intercontinental, intra-continental, and interregional)<sup>[1]</sup>.

Several factors contribute to migration. At the beginning of the 19<sup>th</sup> century, migrants were mostly farmers, farmhands, workmen and refugees but lately, people belonging to the educated and well-off class have started to migrate towards developed countries because of the industrial globalization and the availability of greater opportunities in open-market economies, favored by the Internet revolution<sup>[2]</sup>. These two sets of factors, "push factors" in native countries (food shortage, wars, civil wars, terrorism) and "pull factors" in host countries (economic booming, job opportunities, well-being) remain the basis of migration.

Massive immigration has generated a series of clinical-administrative problems to National Health Systems throughout the world, and also to universalistic systems. This is mainly the case with the so-called chronic non-communicable diseases (NCD), particularly diabetes, due to the very high number of cases at risk<sup>[3]</sup>, the long duration of treatment, the very high cost of complications<sup>[4]</sup>, as well as the availability of effective preventive and therapeutic measures expected to alleviate the burden of disease<sup>[5]</sup>.

We reviewed the documents of International Agencies on migration and performed a PubMed search of existing literature, using the terms "diabetes" and "migrants". This manuscript is aimed at pinpointing the problems arising in the worldwide prevention and treatment of diabetes, with specific reference to countries where they have been more extensively investigated, as a support to healthcare systems in the provision of effective interventions.

### **A global view of migration**

On a worldwide scale, the total number of economic

migrants exceeds 200 million, not considering asylum-seeking refugees. Albeit slowed down in the recent phase of recession, the flow will keep on in Europe and in Asia too, where China will become the main pole of attraction of the migration scenario<sup>[2]</sup>. Only about 10%-15% of migrants from all over the world are in an irregular situation: most of them have entered in a legal way but remain more long-term than their authorized residence<sup>[6]</sup>.

As of January 2014, the number of migrants for economic reasons in the 28 countries of the European Union (EU) totals 19.6 million, representing 3.9% of the population (Figure 1). In absolute terms, the largest numbers are found in Germany (7.0 million persons), the United Kingdom (5.0 million), Italy (4.9 million), Spain (4.7 million) and France (4.2 million), collectively representing 76% of the total. Besides, according to estimates, there is an unknown number of undocumented migrants, accounting for a huge proportion of the population (up to 4%)<sup>[7]</sup>. The total number is expected to increase dramatically in the near future, due to massive migration of asylum-seekers refugees from conflict areas in Middle East and Northern Africa. A few EU countries are facing for the first time the problem of immigration and multi-ethnic population. Migrants have long been a negligible problem in Italy. In 1991, for the first time the number of migrants exceeded 1% of the total Italian population. Later, the number doubled in ten years to reach 7.5% by the end of 2010<sup>[8]</sup>.

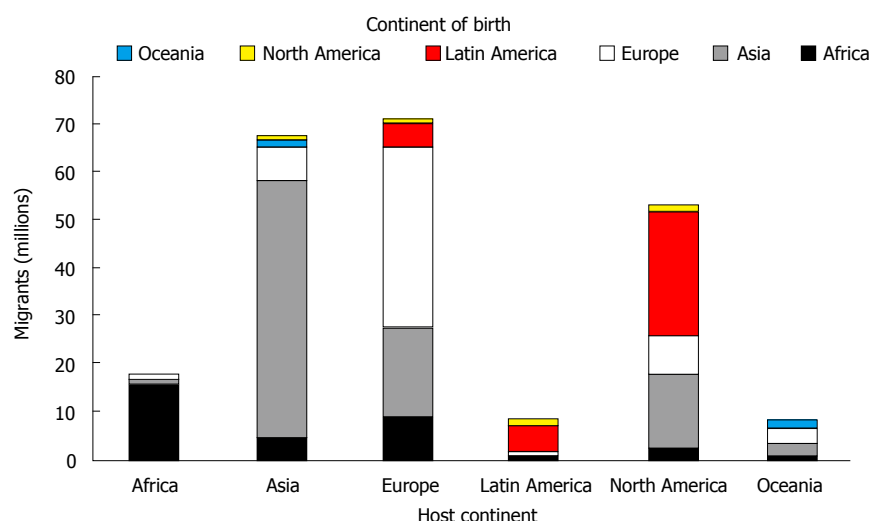
Migration to the United States has also been increasing since 1945 with the current immigrant population estimated at 38.5 million or 12% of the total population<sup>[9]</sup> (Figure 1). Large waves of immigration occurred over the past 20 years<sup>[10]</sup>; by 2050, nearly 1 in 5 United States residents is expected to be an immigrant, compared with 1 in 8 in 2005<sup>[11]</sup>.

Since 1945, more than 7 million people have also settled in Australia. The 2011 Census reported that over one in four of Australian 22-million people were born overseas. Most of them were born in New Zealand (16.2%), the United Kingdom (13.6%), India (10.9%), China (10.0%) and South Africa (4.6%) (Figure 1). Pacific Islanders (Samoans and Tongans) represent another large component of immigration<sup>[12]</sup>.

The complexity of migration remains a fundamental problem. Most statistics on migration are based on concepts that are not representative of a good deal of the dynamics of today migration flows. It is nearly impossible to have the exact numbers of short-term movements and status, as well as to assess properly the extent of undocumented migration that exploded around and across the Mediterranean Sea.

### **Problems in the study of chronic NCD in migrants**

Different data sources may be used to describe the outbreak of NCDs in relation to indicators of migration or ethnicity, as country of birth, self-identified ethnicity, as well as more specific features (language and religious affiliation)<sup>[13-15]</sup>. The essence of ethnicity implies same origins



**Figure 1** Worldwide numbers of migrants (over 200 million) in relation to continent of birth and host continent. Note that most migration occurred intra-continentially. Massive inter-continental migration is recorded from Africa, Asia and Latin America to Europe, as well as from Asia and Latin America to North America, with virtually no migrants to Africa and Latin America and from Oceania.

or social environment, definite culture and customs, and a common language or religious heritage<sup>[16,17]</sup>, but proxy measures are difficult to define. Country of birth is a crude method, which becomes a vague measure as time since migration goes on<sup>[15,18]</sup>. Nationality or citizenship represent critical indexes as immigrants may have nationality and citizenship of the host country, but yet belong to ethnic groups with different lifestyle habits, religion and culture. Without an overall agreement concerning both the definition of ethnicity and of being a migrant, it is difficult to set the reasons behind the prevalence of NCDs or diabetes. The relation between migration status and disease may be affected by genes, exposure in pre-migration life to poverty, precarious health and sanitary conditions, eating habits, infections, rooted cultural customs retained in adulthood, exposures in post-migration life, as well as quality of health care and access to health services in countries of destination (Figure 2).

### Diabetes prevalence in migrants vs native populations

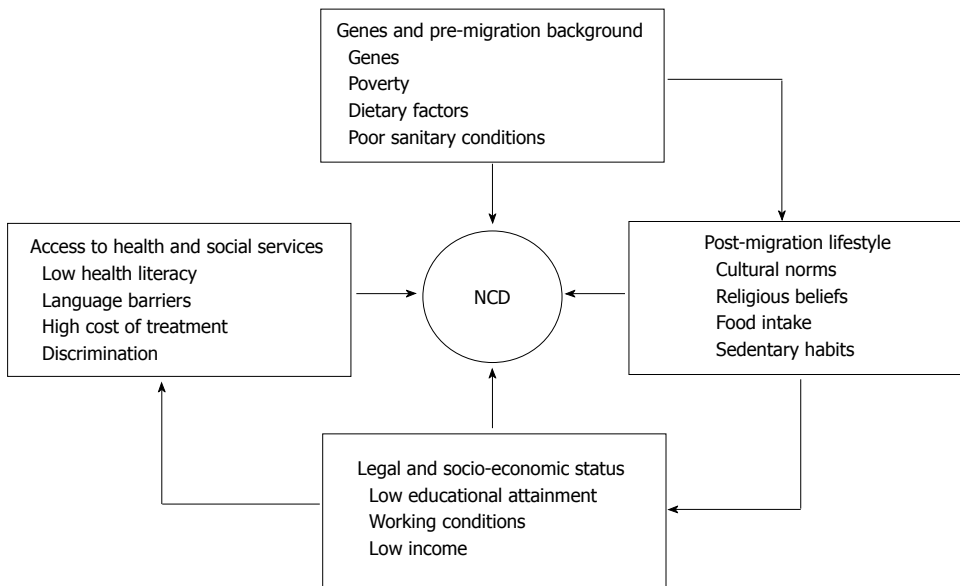
**Europe:** With a few exceptions, prevalence, incidence and mortality rates for diabetes are much higher in migrants than in native people<sup>[2,19-22]</sup>. In the Netherlands, diabetes is more common (by a factor of 2) among the main groups of immigrants, *i.e.*, those born in Turkey, Morocco, Suriname or the Antilles. Even larger differences are observed in diabetes-related mortality, with rates 3 and 4 times higher among migrant men and women, respectively, compared to the indigenous population. Surinamese migrants have the highest prevalence and mortality rates<sup>[23]</sup>, which are most likely due to the higher incidence, although differences in case-fatality rates may also exist.

In the United Kingdom, the prevalence of diabetes mellitus among migrants of South Asian origin (Afghanistan, Bhutan, Maldives, Nepal and Sri Lanka) is around 20%, *i.e.*, nearly five times higher than the local European population. Furthermore, age at onset of diabetes is 5-10-year earlier and chronic complications are more common amongst migrant populations<sup>[24]</sup>. In Italy, the prevalence of diabetes

in migrants is definitely lower than in the general population, but only 15% of migrants are less than 50, vs 43.1% of the Italian population<sup>[25]</sup>. When adjusted for age and sex in a case-control study, the overall risk of diabetes in migrants was 1.55 (95%CI: 1.50-1.60)<sup>[26]</sup>. Notably, the risk varies among ethnic groups; the likelihood of being treated with a glucose-lowering drug is four-fold higher in people from Egypt and the Indian subcontinent, whereas it is halved in migrants from former Eastern socialist countries, in keeping with diabetes prevalence in their countries of origin<sup>[13]</sup>. Also in African migrants to France diabetes develops earlier compared to those staying their country of birth<sup>[27]</sup>.

A 20-year longitudinal follow-up of first generation migrants residing in the United Kingdom reports an incidence of type 2 diabetes almost 3 times higher in the Indian Asian population and more than twice in the African Caribbeans, compared with the European controls. Notably, in the female population the increased likelihood of having diabetes was attributed to baseline insulin resistance and abdominal adiposity, not in men<sup>[28]</sup>. The Healthy Life in an Urban Setting (HELIUS) study, started in 2011, aimed at assessing the factors contributing to the occurrence of diseases, including NCD, cardiovascular diseases and mental disorders, in association with ethnic differences, in a cohort of about 60000 Amsterdam residents representative of 5 migrant groups as well as native residents<sup>[29]</sup>. Patients with diabetes coming from Asia, Middle East and Sub-Saharan Africa if compared to Western populations are at particularly higher risk of microvascular complications, *i.e.*, diabetic retinopathy, nephropathy and peripheral neuropathy<sup>[30]</sup>. It is also the case in ethnic minorities of the same regions settled in Western countries; *e.g.*, in the United Kingdom minority ethnic communities with type 2 diabetes, compared to white Europeans, are more likely to develop ocular complications, including sight-threatening retinopathy and maculopathy<sup>[31]</sup>.

In seven European countries diabetes mortality of 30 migrant groups was much higher than in native residents (almost 90% higher for the male population and 120% higher for the female population)<sup>[32]</sup>. An English follow-



**Figure 2 Factors associated with non-communicable diseases in migrants.** A complex interaction between genetic, cultural and socio-economic factors is the basis for the development of NCDs, variably associated in different ethnic groups. NCD: Non-communicable diseases.

up study reported higher diabetes mortality rates in patients from South Asia compared to European patients, particularly among the young population<sup>[33]</sup>. Similar observations have been made amongst Asian Indian migrants<sup>[34]</sup>.

These differences might be ascribed to a genetic background. Nevertheless, the few studies considering socio-economic factors and quality of diabetes care show a reduction of the role of ethnic differences in complication rates. Therefore, complications among ethnic minority groups with diabetes might also be driven by failure to achieve treatment goals and/or lower screening rates and preventive measures<sup>[31]</sup>.

**United States:** In 9 regions of birth, covering a hundred countries, representing 16 million United States immigrants, overweight/obesity and diabetes prevalence were regularly estimated and compared from 1997 to 2005. Most of migrants were born in Mexico (48%), followed in order of decreasing number by migrants from all Asian regions (almost 20%), Europe, South America, Africa, the Middle East, and Russia. Among United States immigrants, a substantial heterogeneity is noted, by region of birth, in the prevalence of both diabetes and overweight, with diabetes rates stretching from 3.1% in Europe to 10.0% in the Indian subcontinent<sup>[35]</sup>. Migrants from South America, generally considered as Hispanic ethnicity, have lower diabetes and overweight prevalence than migrants from Mexico, Central America, and the Caribbean Islands; *i.e.*, gathering individuals by ethnicity may conceal important differences in the prevalence of the disease.

Blacks (without any distinction for immigrant status) have higher rates of diabetes compared with whites<sup>[36]</sup>. The difference is likely to stem from the increased insulin resistance of black people at adiposity levels similar to

whites<sup>[37]</sup>, particularly in African migrant men, not in women. Black women have twice the obesity prevalence of white women<sup>[38]</sup>, and a prospective incidence study on ethnicity and diabetes in middle-aged adults, African American men and, to a lesser extent, women had a considerably higher incidence of type 2 diabetes compared with white people. The elevated incidence and prevalence of diabetes is thus explained by modifiable risk factors such as adiposity<sup>[39]</sup>.

The three largest Asian-American, native Hawaiian, Pacific Islander subgroups [people of Chinese (3.3 million), South Asian (2.8 million), or Filipino (2.6 million) ancestry] are all at increased risk to develop diabetes<sup>[40]</sup>. In general, their risk is higher relative to non-Hispanic whites, but lower than that of African Americans and Latinos<sup>[41,42]</sup>. Although data are limited<sup>[43]</sup>, diabetes prevalence is more than doubled among Pacific Islanders (18.3%) vs with white participants (7.3%), and significantly higher than among other Asian subgroups, confirming that continental data must be disaggregated on a national scale<sup>[42]</sup>. Ethnicity-specific risks of micro-vascular complications (retinopathy) have also been demonstrated<sup>[44]</sup>.

**Australia:** For all migrant groups, the odds of type 2 diabetes vs native residents are higher, after adjusting for age and across all socio-economic strata<sup>[45]</sup>. In the Fremantle Diabetes Study, the prevalence in Asians and the general population was similar, but the Asian patients were younger, less obese and less likely to be hypertensive. Nonetheless, they had a higher prevalence of retinopathy. During an 18-year follow-up, Asian ethnicity was independently protective against cardiovascular death, not all-cause mortality<sup>[46]</sup>. According to the Melbourne Collaborative Cohort Study<sup>[47]</sup>, the baseline prevalence and the cumulative incidence of type 2 diabetes were more



than three-fold higher in migrants born in Greece or Italy than in individuals born in Australia<sup>[48]</sup>. These findings are consistent with the higher prevalence showed by Australian cross-sectional studies<sup>[49,50]</sup>.

Higher BMI in the migrants was responsible for almost one-half the excess relative risk in incidence, whereas other risk factors for diabetes, including the waist-to-hip ratio, and diet, had little impact on the remaining excess relative risk. However, there is no evidence for a specific genetic susceptibility to diabetes in Italian migrants<sup>[51]</sup>. Health care is universally available in Australia and generally of good standard. Thus, the risk of excess mortality in migrants because of different chances of access to treatment and standard of care is minimized. The poorer outcome of migrant people with diabetes remains a priority study area, subject to continuous scrutiny<sup>[52]</sup>.

### **Prevalence of NCD in migrants vs rates in the countries of origin**

When the prevalence of diabetes in migrants is compared with that in the country of origin, the general characteristics, the prevalence of obesity, as well as the general degree of socioeconomic development, as measured by the gross domestic product<sup>[32]</sup>, should always be considered. The lower the socio-economic status in the country of origin, the higher the risk to become obese. For example, in a study investigating on Ghanaian migrants in the Netherlands, people living in Amsterdam compared to those living in rural Ghana<sup>[53]</sup>, were 10 times more likely to be overweight, and overweight represents a risk factor for the development of diabetes. This explains why among migrants from low-income countries compared to locally born European populations, diabetes mortality rates are more than 200% higher, in comparison to a 100% higher rates for migrants from middle-income countries<sup>[32]</sup>. In view of these considerations the high diabetes mortality of migrants seems to be associated to the movement from a poverty-ridden rural area, in early life, to an obesogenic urban environment in later life, with a few exceptions.

According to previous researches Asian Indians who moved to the United States have worse metabolic profiles when compared to their counterparts still living in India<sup>[54]</sup>. Contrary to that, the CARRS and MASALA Studies<sup>[55]</sup> reported a higher diabetes prevalence in Indian people living in India than their homologous who have migrated to the United States, even with Asian Indians living in India having lower BMI and lower waist circumference values than their counterparts who have migrated to the United States. Surprisingly, both the overall and the age specific prevalence of prediabetes resulted lower in Asian Indians living in India than in Asian Indians minorities who moved to the United States; the explication could be linked to a more rapid conversion, through the natural history of the disease, in people living in India. Furthermore, the prevalence of type 2 diabetes in Asian Indians living in the United States was still significantly higher than that of the

general United States population<sup>[41,55,56]</sup>, despite the fact that the general United States population has, compared to Asian Indians, an overall higher BMI. Therefore it could be probable that India finds itself in an early stage of the diabetes epidemic; thus most susceptible subjects develop it the earliest<sup>[57]</sup>.

Having regard to the current phase of rapid economic and nutritional changes over the Indian continent<sup>[58,59]</sup>, these aspects could increase the risk in Asian Indians both in India and abroad. It is also likely that Asian Indians who have migrated to the United States have adopted healthier lifestyle habits, both in food choices and physical exercise, reducing their risk of type 2 diabetes occurrence<sup>[60]</sup>, thereby altering the relation existing in this population between migration and NCD risk.

Another way of analyzing the role of country of origin is to compare migrants who come from the same country, now living in different countries. From January 2012, a multicenter cross-sectional study is evaluating differences in lifestyle, epigenetics and biochemistry that increase the risk of type 2 diabetes and obesity among homogenous sub-Saharan African participants (*i.e.*, Ghanaians) aged > 25 years living in rural and urban Ghana, the Netherlands, Germany and the United Kingdom<sup>[61]</sup>.

Genetic factors should anyway be considered to dictate the prevalence of NCD in migrants, as compared to the native populations. Genetic studies have confirmed a role of the ethnic background in the higher prevalence of diabetes in South Asian people<sup>[62-64]</sup> and in the higher rates of hypertension among West African migrants<sup>[65,66]</sup>, whereas the obesogenic environment of the country of immigration makes the difference between NCD prevalence in migrants vs the corresponding prevalence in the country of origin.

### **Disease convergence**

In the long-term, diabetes risk in migrants is expected to converge towards the levels of the locally born populations. This relates to the "healthy migrant" effect, *i.e.*, migration is expected to be a selective process favoring healthy individuals, resulting in low occurrence of diseases among migrants in the first period after their arrival in the host countries<sup>[67]</sup>. As an example, in 2013, the median age of the national population in the EU-28 was 43 years, while the median age of non-nationals living in the EU was 35 years. This protective effect is also present with other NCDs<sup>[46]</sup>, but as time goes by the relative advantage of migrants over locally born subjects could reduce.

Convergence can be also predicted due to the fact that migrants generally integrate over time into the society, and its customs, of their host country<sup>[68]</sup>. As migrants adopt the same habits being exposed to the same environmental risk factors as indigenous people, albeit gradually and slowly<sup>[69-71]</sup>, their epidemiological profiles may move ever closer to those of locally born

subjects<sup>[68]</sup>. "Period" studies (comparing different time periods)<sup>[70,72,73]</sup> and "cohort" studies (comparing cohorts of migrants differing in terms of acculturation)<sup>[69]</sup> supported the convergence hypothesis: The risk of cancer resulted more similar to that of the indigenous populations among people with a longer period of migration, *i.e.*, among the second generation, and, within the first generation, among people who had migrated during infancy. However, convergence takes a lot of time, more than 20 years in some studies<sup>[74]</sup>.

Another theory explains lower mortality in migrants by "the salmon bias" hypothesis, reflecting the trend of sick migrants returning back to their home country before death; in most settings, this does not fully explain the paradox<sup>[67,75,76]</sup>, and it is possible that inaccurate counting of mortality among migrants minority groups lead to an underestimation bias<sup>[77]</sup>.

### **Diabetes risk, disease progression and diabetes care in migrants**

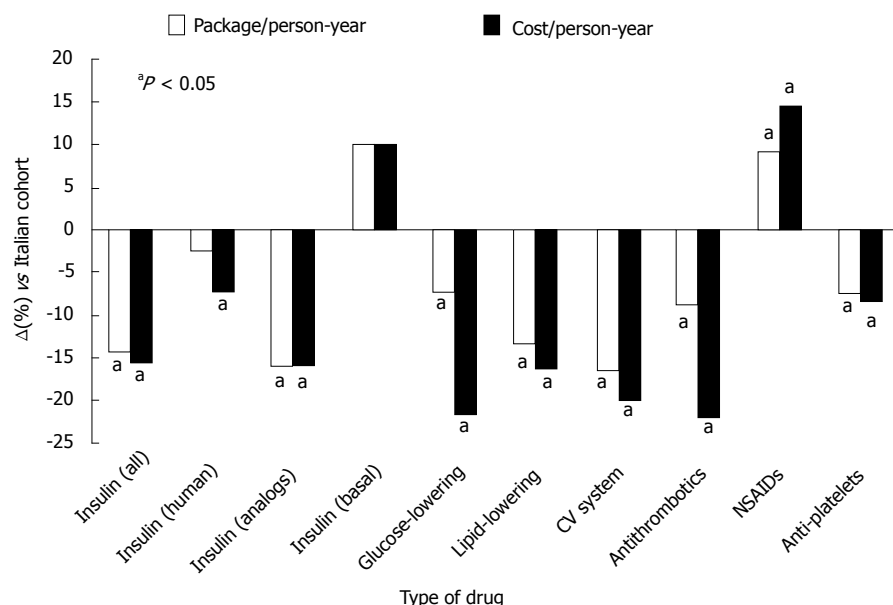
Predisposition to develop insulin resistance and truncal obesity, the exposure to a particular intrauterine environment and even the biological imprinting all push in the same direction enhancing the risk of diabetes<sup>[78]</sup>, as well as its progression, in migrant populations, frequently fuelled by differences in standard of care. Many migrants in non-Western countries have risen in conditions of poverty and their bodies have been "programmed" to tackle hunger and starvation. The result is that later in life, when exposed to the obesogenic environment of the country of immigration (high-fat diet and sedentary lifestyle), they are particularly prone to stock energy reserves and thereby gain weight<sup>[2,53]</sup>. A cross-sectional study in a large cohort of migrants from the Philippines to the city of Rome recorded abdominal obesity in 52.5% and a high prevalence of undiagnosed type 2 diabetes and hypertension<sup>[79]</sup>. Years of residence in Italy showed a significant direct correlation with the degree of changes in food intake ( $P = 0.001$ ) and weight gain ( $P < 0.001$ ), suggesting a direct impact of lifestyle.

Also the so-called social determinants of health may be involved (*i.e.*, low socio-economic status, separation from family, anti-migrant feelings in the host community, traditional beliefs influencing the health-seeking behavior, the lack of legislation to ensure migrants' access to health and social services, effective policies to protect migrants' labor rights and welfare). This is mainly the case with insurance-type health care systems, but poor access to health services is also demonstrated in health care systems that are essentially built on a principle of equity and equality. Integration of migrant population into the receiving healthcare system is becoming a key issue in developed countries who receive a large number of migrants each year. Independently of the healthcare system, several reports have shown that migrants are undertreated compared with the native population<sup>[80,81]</sup>. This is probably related to a lower-than-needed attendance of migrants to primary care or preventive health services, due to personal barriers (job and time

constraints) or socio-cultural conditions, not only to specific defects in the healthcare system<sup>[82,83]</sup>. In native Dutch and in various groups of immigrants aged 55 and older, the use of prescribed drugs was explained by Andersen's behavioral model<sup>[84]</sup>, based on three individual factors of health care use: (1) need (self-rated chronic conditions); (2) enabling conditions (educational level, standardized household income); and (3) predisposing factors (Dutch language proficiency, modern attitudes on family care, male-female roles, family values, religion)<sup>[85]</sup>. Differences in the verbal interaction of Dutch GPs with immigrant vs Dutch patients have been demonstrated by video recording: Consultations with immigrant patients were over 2 min shorter, with major differences in verbal interaction and less empathy<sup>[82]</sup>. In diabetes, language proficiency and modern attitudes on male-female roles have been associated with utilization of diabetes drugs, especially in Turkish<sup>[86]</sup> and Moroccan elderly patients<sup>[85]</sup>, where inequalities might be amplified by behavioral components (smoking, inactivity, alcohol consumption)<sup>[87,88]</sup>.

Leisure time physical activity is definitely lower in migrants<sup>[89-91]</sup>, but the amount of calories spent at work, because of more common manual labor, might be considerably higher<sup>[64]</sup>. An Australian study reported a higher relative risk of smoking and alcohol use<sup>[92]</sup>, and lower adherence to recommendation for healthy diet is also frequently described. A systematic review from the United Kingdom, on a migrant South Asian population, reported that all South Asian groups were found to have a more sedentary lifestyle than their European counterparts<sup>[93]</sup>. However, the high variability and the large proportion of different migrant minorities moving across the world make it difficult to draw any conclusions.

Inequalities in migrant health care are not limited to diabetes groups. In a Swiss cohort of forced, asylum seekers migrants, lower scores for physical examinations and breast and colon cancer screening have been reported, despite universal healthcare coverage<sup>[94]</sup>. In a Swedish population-based follow-up concerning drugs given to patients after acute myocardial infarction, no major differences were observed in relation to socio-economic status<sup>[95]</sup>. However, among immigrants from outside the EU countries there was a 20%-30% reduced prescription of drugs recommended by Swedish guidelines (aspirin, beta-blockers, cholesterol-lowering drugs and ACE inhibitors). Also in this case, ethnicity may make the difference; in ethnic German migrants from the former Soviet Union to Germany only minor differences in drug utilization pattern were recorded, compared with native Germans<sup>[96]</sup>. In this case, however, much similar habits and traditions might help canceling the differences. Far from being money-saving for the Health Systems, underutilization of drugs and primary care services in specific ethnic groups is expected to produce a larger-than-needed use of emergency services and hospitalization rates due to disease progression and complications, which might translate into higher costs<sup>[97,98]</sup>.



**Figure 3** Differences in drug use and overall pharmacologic cost of diabetes in migrants, compared with native Italians. Both drug use and total costs were lower in migrants, with a different use of insulin and oral hypoglycemic agents. Non-steroidal anti-inflammatory drug use was higher, possibly as a consequence of more common traumas and manual work.

**Data from the ARNO database:** Drug use and the direct costs of drug-treated diabetes among migrants and Italian citizens has been extensively investigated on the basis of 2010 prescriptions in the population-based multiregional ARNO Observatory, a database containing the prescriptions and hospital admissions of a population of about 10 million Italian residents, living in 30 Health districts scattered throughout the country<sup>[99]</sup>. According to Italian rules, a diagnosis of diabetes grants free access to drugs, diagnostic procedures and hospitalization to persons residents in Italy, independently of their citizenship. All drug prescription data or hospital admission records contain a code including the date and place of birth. For people born outside Italy, the city code is replaced by the country code, thus permitting univocal identification of people born outside Italy.

Based on the above criteria, in the nested ARNO population of over 8 million people first and second-generation migrants were classified according to country of birth and citizenship. All patients who had at least one prescription of anti-diabetic medication, either oral agents or insulin (Anatomical Therapeutic Chemical Classification System, code A10A and A10B, respectively) during 2010 were considered as affected by diabetes. The odds of migrants of being diagnosed with diabetes compared to Italians were tested using a case-control study design, with one migrant matched for major confounders (age, sex and place of residence) to one Italian subject. Finally, migrants with diabetes were individually matched for confounders to Italians with diabetes to compare prescriptions, hospitalization rates, use of services and direct costs for the National Health System.

Migrants with diabetes were 15-year younger than native Italians. Both glucose-lowering and non-glucose-lowering drugs were underused in migrants with diabetes, with the notable exception of a few drugs (*i.e.*, non-steroidal anti-inflammatory agents), whose excess use might be related to more intense manual and traumatic work (Figure 3). Migrants had a different

pattern of glucose-lowering treatment, with 44% higher prescriptions of oral drugs and 19% lower prescriptions of insulin and a different insulin pattern<sup>[26,100]</sup>. Also lipid-lowering drugs and antithrombotic drugs were 15%-20% underused in migrants. The total cost was 27% lower in migrants, due to a lower cost of drugs (29%), hospital admission (27%) and health services (22%)<sup>[26]</sup>.

Notably, hospitalization rates due to diabetes per se were 60% more common in migrants vs native Italians of the ARNO observatory<sup>[26]</sup>. This might represent the consequence of under-treatment, also considering that hospital length-of-stay was longer in migrants. Contrary to that, a report from the London School of Economics found that hospital stay among the immigrant population was significantly shorter in Europe after adjusting for age, case mix and disease severity, suggesting unequal treatment for equal needs<sup>[101]</sup>. A cross-sectional analysis of health service use among elderly immigrants and native populations of 11 European countries recorded a migrants' overutilization of hospital stay and consultations in several countries, not in Italy, due to difficulties in the integration of migrants because of very recent immigration and cultural reasons<sup>[102]</sup>. No data are available on the attendance of migrants to emergency departments not followed by hospital admission, a procedure not traced by the Italian Health System. Emergency services might be overused, fuelled by socio-economic inequalities<sup>[103]</sup>, to provide immediate care to less complicated conditions.

## CONCLUSION

The growing burden of chronic diseases, specifically diabetes, in migrant and ethnic minorities represents a serious public health challenge for many European countries, also fuelled by the economic crisis, social inequalities, terrorism and wars. Migrant flow is not expected to slow down in the next few years, and it will generate an increasing economic cost for the National

Health System. The same is true for United States, where African and Mexican Americans may experience higher rates of diabetes prevalence, which will translate into different and increasing costs.

High-risk migrant minorities may remain undertreated and unprotected also in a universalistic healthcare system<sup>[104]</sup>. Poorer socioeconomic status and barriers generated by the present culture of biomedicine might make the difference on patients' side, but inequalities might also stem from physicians' side, with a different approach by both primary care physicians and specialists<sup>[105]</sup>. Efforts should be made to favor visits to general practitioners/diabetologists and attendance to screening and treatment programs, to adapt education programs to specific cultures<sup>[106]</sup>, to cope with misbeliefs<sup>[107]</sup>, and to develop community partnerships.

## REFERENCES

- 1 **United Nations Statistics Division.** Recommendations on Statistics of International Migration, Revision 1. Geneva, United Nations, 1998. Available from: URL: <http://unstats.un.org/unsd/iiss/Recommendations-on-Statistics-of-International-Migration-Revision-1.ashx>
- 2 **Misra A, Ganda OP.** Migration and its impact on adiposity and type 2 diabetes. *Nutrition* 2007; **23**: 696-708 [PMID: 17679049 DOI: 10.1016/j.nut.2007.06.008]
- 3 **Hempler NF, Diderichsen F, Larsen FB, Ladelund S, Jørgensen T.** Do immigrants from Turkey, Pakistan and Yugoslavia receive adequate medical treatment with beta-blockers and statins after acute myocardial infarction compared with Danish-born residents? A register-based follow-up study. *Eur J Clin Pharmacol* 2010; **66**: 735-742 [PMID: 20393695 DOI: 10.1007/s00228-010-0816-3]
- 4 **American Diabetes Association.** Economic costs of diabetes in the U.S. in 2012. *Diabetes Care* 2013; **36**: 1033-1046 [PMID: 23468086 DOI: 10.2337/dc12-2625]
- 5 **Nicolucci A, Rossi MC, Arcangeli A, Cimino A, de Gigontina G, Fava D, Gentile S, Giorda C, Meloncelli I, Pellegrini F, Valentini U, Vespasiani G.** Four-year impact of a continuous quality improvement effort implemented by a network of diabetes outpatient clinics: the AMD-Annals initiative. *Diabet Med* 2010; **27**: 1041-1048 [PMID: 20722678 DOI: 10.1111/j.1464-5491.2010.03055.x]
- 6 **International Organization for Migration.** World Migration Report 2010. Geneva, International Organization for Migration, 2010. Available from: URL: [http://publications.iom.int/system/files/pdf/wmr\\_2010\\_english.pdf](http://publications.iom.int/system/files/pdf/wmr_2010_english.pdf)
- 7 **Karl-Trummer U, Metzler B, Novak-Zezula S.** Health Care for Undocumented Migrants in the EU: Concepts and Cases. Brussels, IOM, 2009. Available from: URL: [http://www.migrant-health-europe.org/files/Health\\_Care\\_for\\_Undocumented\\_Migrants\\_Background\\_Paper\(6\).pdf](http://www.migrant-health-europe.org/files/Health_Care_for_Undocumented_Migrants_Background_Paper(6).pdf)
- 8 **Caritas/Migrantes.** Dossier Statistico Immigrazione. Rome, IDOS - Centro Studi e Ricerche, 2011. Available from: URL: <http://www.dossierimmigrazione.it/docnews/file/pres2011-scheda.pdf>
- 9 **US Census Bureau.** The 2009 American Community Survey. Washington, DC, US Census Bureau, 2010. Available from: URL: [https://www.census.gov/newsroom/releases/archives/american\\_community\\_survey\\_acs/cb10-cn78.html](https://www.census.gov/newsroom/releases/archives/american_community_survey_acs/cb10-cn78.html)
- 10 **Kandula NR, Kersey M, Lurie N.** Assuring the health of immigrants: what the leading health indicators tell us. *Annu Rev Public Health* 2004; **25**: 357-376 [PMID: 15015925 DOI: 10.1146/annurev.publhealth.25.101802.123107]
- 11 **Passell JS, Cohn D.** US Population Projections: 2005-2050. Washington, DC: Pew Research Center, 2008. Available from: URL: <http://www.pewsocialtrends.org/files/2010/10/85.pdf>
- 12 **Statistics New Zealand and Ministry of Pacific Affairs.** Demographics of New Zealand's Pacific population. Wellington, Statistics New Zealand, 2010. Available from: URL: [http://www.stats.govt.nz/browse\\_for\\_stats/people\\_and\\_communities/pacific\\_peoples/pacific-progress-demography.aspx](http://www.stats.govt.nz/browse_for_stats/people_and_communities/pacific_peoples/pacific-progress-demography.aspx)
- 13 **Rafnsson SB, Bhopal RS.** Large-scale epidemiological data on cardiovascular diseases and diabetes in migrant and ethnic minority groups in Europe. *Eur J Public Health* 2009; **19**: 484-491 [PMID: 19498046 DOI: 10.1093/eurpub/ckp073]
- 14 **Stronks K, Kunst AE.** The complex interrelationship between ethnic and socio-economic inequalities in health. *J Public Health (Oxf)* 2009; **31**: 324-325 [PMID: 19589801 DOI: 10.1093/pubmed/fdp070]
- 15 **Stronks K, Kulu-Glasgow I, Agyemang C.** The utility of 'country of birth' for the classification of ethnic groups in health research: the Dutch experience. *Ethn Health* 2009; **14**: 255-269 [PMID: 19052941 DOI: 10.1080/13557850802509206]
- 16 **Lin SS, Kelsey JL.** Use of race and ethnicity in epidemiologic research: concepts, methodological issues, and suggestions for research. *Epidemiol Rev* 2000; **22**: 187-202 [PMID: 11218371]
- 17 **Ford ME, Kelly PA.** Conceptualizing and categorizing race and ethnicity in health services research. *Health Serv Res* 2005; **40**: 1658-1675 [PMID: 16179001 DOI: 10.1111/j.1475-6773.2005.00449.x]
- 18 **Gill PS, Bhopal R, Wild S, Kai J.** Limitations and potential of country of birth as proxy for ethnic group. *BMJ* 2005; **330**: 196 [PMID: 15661790 DOI: 10.1136/bmj.330.7484.196-a]
- 19 **Deboosere P, Gadeyne S.** Adult migrant mortality advantage in Belgium: evidence using census and register data. *Population* 2005; **60**: 655-698
- 20 **Kristensen JK, Bak JF, Wittrup I, Lauritzen T.** Diabetes prevalence and quality of diabetes care among Lebanese or Turkish immigrants compared to a native Danish population. *Prim Care Diabetes* 2007; **1**: 159-165 [PMID: 18632038 DOI: 10.1016/j.pcd.2007.07.007]
- 21 **Ujcic-Voortman JK, Schram MT, Jacobs-van der Bruggen MA, Verhoeff AP, Baan CA.** Diabetes prevalence and risk factors among ethnic minorities. *Eur J Public Health* 2009; **19**: 511-515 [PMID: 19587231 DOI: 10.1093/eurpub/ckp096]
- 22 **Jenum AK, Diep LM, Holmboe-Ottesen G, Holme IM, Kumar BN, Birkeland KI.** Diabetes susceptibility in ethnic minority groups from Turkey, Vietnam, Sri Lanka and Pakistan compared with Norwegians - the association with adiposity is strongest for ethnic minority women. *BMC Public Health* 2012; **12**: 150 [PMID: 22380873 DOI: 10.1186/1471-2458-12-150]
- 23 **Stirbu I, Kunst AE, Bos V, Mackenbach JP.** Differences in avoidable mortality between migrants and the native Dutch in The Netherlands. *BMC Public Health* 2006; **6**: 78 [PMID: 16566833 DOI: 10.1186/1471-2458-6-78]
- 24 **Gholap N, Davies M, Patel K, Sattar N, Khunti K.** Type 2 diabetes and cardiovascular disease in South Asians. *Prim Care Diabetes* 2011; **5**: 45-56 [PMID: 20869934 DOI: 10.1016/j.pcd.2010.08.002]
- 25 **CINECA.** Osservatorio ARNO Diabete: il profilo assistenziale della popolazione con diabete Bologna, Centauro Srl - Edizioni Scientifiche, 2011. Available from: URL: <https://osservatorioarno.cineca.org/diabete/razionale2011.htm>
- 26 **Marchesini G, Bernardi D, Miccoli R, Rossi E, Vaccaro O, De Rosa M, Bonora E, Bruno G.** Under-treatment of migrants with diabetes in a universalistic health care system: the ARNO Observatory. *Nutr Metab Cardiovasc Dis* 2014; **24**: 393-399 [PMID: 24462046 DOI: 10.1016/j.numecd.2013.09.012]
- 27 **Choukem SP, Fabreguettes C, Akwo E, Porcher R, Nguewa JL, Bouche C, Kaze FF, Kengne AP, Vexiau P, Mbanya JC, Sobngwi E, Gautier JF.** Influence of migration on characteristics of type 2 diabetes in sub-Saharan Africans. *Diabetes Metab* 2014; **40**: 56-60 [PMID: 24076360 DOI: 10.1016/j.diabet.2013.07.004]
- 28 **Tillin T, Hughes AD, Godsland IF, Whincup P, Forouhi NG, Welsh P, Sattar N, McKeigue PM, Chaturvedi N.** Insulin resistance and truncal obesity as important determinants of the greater incidence of diabetes in Indian Asians and African Caribbeans compared with Europeans: the Southall And Brent REvisited (SABRE) cohort. *Diabetes Care* 2013; **36**: 383-393 [PMID: 22966089 DOI: 10.2337/131111]



- 10.2337/dc12-0544]
- 29 **Stronks K**, Snijder MB, Peters RJ, Prins M, Schene AH, Zwinderman AH. Unravelling the impact of ethnicity on health in Europe: the HELIUS study. *BMC Public Health* 2013; **13**: 402 [PMID: 23621920 DOI: 10.1186/1471-2458-13-402]
- 30 **Sivaprasad S**, Gupta B, Gulliford MC, Dodhia H, Mohamed M, Nagi D, Evans JR. Ethnic variations in the prevalence of diabetic retinopathy in people with diabetes attending screening in the United Kingdom (DRIVE UK). *PLoS One* 2012; **7**: e32182 [PMID: 22412857 DOI: 10.1371/journal.pone.0032182]
- 31 **Davis TM**, Coleman RL, Holman RR. Ethnicity and long-term vascular outcomes in Type 2 diabetes: a prospective observational study (UKPDS 83). *Diabet Med* 2014; **31**: 200-207 [PMID: 24267048 DOI: 10.1111/dme.12353]
- 32 **Vandenheede H**, Deboosere P, Stirbu I, Agyemang CO, Harding S, Juel K, Rafnsson SB, Regidor E, Rey G, Rosato M, Mackenbach JP, Kunst AE. Migrant mortality from diabetes mellitus across Europe: the importance of socio-economic change. *Eur J Epidemiol* 2012; **27**: 109-117 [PMID: 22167294 DOI: 10.1007/s10654-011-9638-6]
- 33 **Mather HM**, Chaturvedi N, Fuller JH. Mortality and morbidity from diabetes in South Asians and Europeans: 11-year follow-up of the Southall Diabetes Survey, London, UK. *Diabet Med* 1998; **15**: 53-59 [PMID: 9472864 DOI: 10.1002/(SICI)1096-9136(199801)15:1<53::AID-DIA521>3.0.CO;2-V]
- 34 **Zimmet PZ**, McCarty DJ, de Courten MP. The global epidemiology of non-insulin-dependent diabetes mellitus and the metabolic syndrome. *J Diabetes Complications* 1997; **11**: 60-68 [PMID: 9101389]
- 35 **Oza-Frank R**, Narayan KM. Overweight and diabetes prevalence among US immigrants. *Am J Public Health* 2010; **100**: 661-668 [PMID: 19608956 DOI: 10.2105/AJPH.2008.149492]
- 36 **Department of Health and Human Services, Centers for Disease Control and Prevention**. National Diabetes Fact Sheet, 2007. Available from: URL: <http://search.cdc.gov/search?query=diabetes+fact+sheet&utf8=&affiliate=cdc-main>
- 37 **Okosun IS**, Liao Y, Rotimi CN, Prewitt TE, Cooper RS. Abdominal adiposity and clustering of multiple metabolic syndrome in White, Black and Hispanic americans. *Ann Epidemiol* 2000; **10**: 263-270 [PMID: 10942873]
- 38 **Flegal KM**, Carroll MD, Ogden CL, Johnson CL. Prevalence and trends in obesity among US adults, 1999-2000. *JAMA* 2002; **288**: 1723-1727 [PMID: 12365955]
- 39 **Brancati FL**, Kao WH, Folsom AR, Watson RL, Szklo M. Incident type 2 diabetes mellitus in African American and white adults: the Atherosclerosis Risk in Communities Study. *JAMA* 2000; **283**: 2253-2259 [PMID: 10807384]
- 40 **King GL**, McNeely MJ, Thorpe LE, Mau ML, Ko J, Liu LL, Sun A, Hsu WC, Chow EA. Understanding and addressing unique needs of diabetes in Asian Americans, native Hawaiians, and Pacific Islanders. *Diabetes Care* 2012; **35**: 1181-1188 [PMID: 22517939 DOI: 10.2337/dc12-0210]
- 41 **Lee JW**, Brancati FL, Yeh HC. Trends in the prevalence of type 2 diabetes in Asians versus whites: results from the United States National Health Interview Survey, 1997-2008. *Diabetes Care* 2011; **34**: 353-357 [PMID: 21216863 DOI: 10.2337/dc10-0746]
- 42 **Karter AJ**, Schillinger D, Adams AS, Moffet HH, Liu J, Adler NE, Kanaya AM. Elevated rates of diabetes in Pacific Islanders and Asian subgroups: The Diabetes Study of Northern California (DISTANCE). *Diabetes Care* 2013; **36**: 574-579 [PMID: 23069837 DOI: 10.2337/dc12-0722]
- 43 **McGarvey ST**, Seiden A. Health, well-being, and social context of Samoan migrant populations. *NAPA Bulletin* 2010; **34**: 213-228
- 44 **Tan ED**, Davis WA, Davis TM. Changes in characteristics and management of Asian and Anglo-Celts with type 2 diabetes over a 15-year period in an urban Australian community: The Fremantle Diabetes Study. *J Diabetes* 2016; **8**: 139-147 [PMID: 25581285 DOI: 10.1111/1753-0407.12267]
- 45 **Abouzeid M**, Philpot B, Janus ED, Coates MJ, Dunbar JA. Type 2 diabetes prevalence varies by socio-economic status within and between migrant groups: analysis and implications for Australia. *BMC Public Health* 2013; **13**: 252 [PMID: 23517376 DOI: 10.1186/1471-2458-13-252]
- 46 **Tan ED**, Davis WA, Davis TM. Characteristics and prognosis of Asian patients with type 2 diabetes from a multi-racial Australian community: the Fremantle Diabetes Study. *Intern Med J* 2013; **43**: 1125-1132 [PMID: 23869413]
- 47 **Hodge AM**, Flicker L, O'Dea K, English DR, Giles GG. Diabetes and ageing in the Melbourne Collaborative Cohort Study (MCCS). *Diabetes Res Clin Pract* 2013; **100**: 398-403 [PMID: 23582874 DOI: 10.1016/j.diabres.2013.03.024]
- 48 **Hodge AM**, English DR, O'Dea K, Giles GG. Increased diabetes incidence in Greek and Italian migrants to Australia: how much can be explained by known risk factors? *Diabetes Care* 2004; **27**: 2330-2334 [PMID: 15451896]
- 49 **Welborn TA**, Knuiman MW, Bartholomew HC, Whittall DE. 1989-90 National Health Survey: prevalence of self-reported diabetes in Australia. *Med J Aust* 1995; **163**: 129-132 [PMID: 7643762]
- 50 **McKay R**, McCarty CA, Taylor HR. Diabetes in Victoria, Australia: the Visual Impairment Project. *Aust N Z J Public Health* 2000; **24**: 565-569 [PMID: 11215002]
- 51 **DECODE Study Group**; European Diabetes Epidemiology Group. Age, body mass index and glucose tolerance in 11 European population-based surveys. *Diabet Med* 2002; **19**: 558-565 [PMID: 12099558]
- 52 **Anikeeva O**, Bi P, Hiller JE, Ryan P, Roder D, Han GS. Trends in migrant mortality rates in Australia 1981-2007: a focus on the National Health Priority Areas other than cancer. *Ethn Health* 2015; **20**: 29-48 [PMID: 24498932 DOI: 10.1080/13557858.2014.883368]
- 53 **Agyemang C**, Owusu-Dabo E, de Jonge A, Martins D, Ogedegbe G, Stronks K. Overweight and obesity among Ghanaian residents in The Netherlands: how do they weigh against their urban and rural counterparts in Ghana? *Public Health Nutr* 2009; **12**: 909-916 [PMID: 18761759 DOI: 10.1017/S1368980008003510]
- 54 **Bhatnagar D**, Anand IS, Durrington PN, Patel DJ, Wander GS, Mackness MI, Creed F, Tomenson B, Chandrashekar Y, Winterbotham M. Coronary risk factors in people from the Indian subcontinent living in west London and their siblings in India. *Lancet* 1995; **345**: 405-409 [PMID: 7853948]
- 55 **Gujral UP**, Narayan KM, Pradeepa RG, Deepa M, Ali MK, Anjana RM, Kandula NR, Mohan V, Kanaya AM. Comparing Type 2 Diabetes, Prediabetes, and Their Associated Risk Factors in Asian Indians in India and in the U.S.: the CARRS and MASALA Studies. *Diabetes Care* 2015; **38**: 1312-1318 [PMID: 25877810 DOI: 10.2337/dc15-0032]
- 56 **Kanaya AM**, Herrington D, Vittinghoff E, Ewing SK, Liu K, Blaha MJ, Dave SS, Qureshi F, Kandula NR. Understanding the high prevalence of diabetes in U.S. south Asians compared with four racial/ethnic groups: the MASALA and MESA studies. *Diabetes Care* 2014; **37**: 1621-1628 [PMID: 24705613 DOI: 10.2337/dc13-2656]
- 57 **Qiao Q**, Hu G, Tuomilehto J, Nakagami T, Balkau B, Borch-Johnsen K, Ramachandran A, Mohan V, Iyer SR, Tominaga M, Kiyohara Y, Kato I, Okubo K, Nagai M, Shibasaki S, Yang Z, Tong Z, Fan Q, Wang B, Chew SK, Tan BY, Heng D, Emmanuel S, Tajima N, Iwamoto Y, Snehalatha C, Vijay V, Kapur A, Dong Y, Nan H, Gao W, Shi H, Fu F. Age- and sex-specific prevalence of diabetes and impaired glucose regulation in 11 Asian cohorts. *Diabetes Care* 2003; **26**: 1770-1780 [PMID: 12766108]
- 58 **Griffiths PL**, Bentley ME. The nutrition transition is underway in India. *J Nutr* 2001; **131**: 2692-2700 [PMID: 11584092]
- 59 **Shetty PS**. Nutrition transition in India. *Public Health Nutr* 2002; **5**: 175-182 [PMID: 12027282 DOI: 10.1079/PHN2001291]
- 60 **Venkatesh S**, Weatherspoon LJ, Kaplowitz SA, Song WO. Acculturation and glycemic control of Asian Indian adults with type 2 diabetes. *J Community Health* 2013; **38**: 78-85 [PMID: 22744164 DOI: 10.1007/s10900-012-9584-6]
- 61 **Agyemang C**, Beune E, Meeks K, Owusu-Dabo E, Agyei-Baffour P, Aikins Ad, Dodoo F, Smeeth L, Addo J, Mockenhaupt FP,

- Amoah SK, Schulze MB, Danquah I, Spranger J, Nicolaou M, Klipstein-Grobusch K, Burr T, Henneman P, Mannens MM, van Straalen JP, Bahendeka S, Zwiderman AH, Kunst AE, Stronks K. Rationale and cross-sectional study design of the Research on Obesity and type 2 Diabetes among African Migrants: the RODAM study. *BMJ Open* 2014; **4**: e004877 [PMID: 24657884 DOI: 10.1136/bmjopen-2014-004877]
- 62 **Chowdhury R**, Narayan KM, Zabetian A, Raj S, Tabassum R. Genetic studies of type 2 diabetes in South Asians: a systematic overview. *Curr Diabetes Rev* 2014; **10**: 258-274 [PMID: 25001234 DOI: 10.2174/1573399810666140707101325]
- 63 **Mahajan A**, Go MJ, Zhang W, Below JE, Gaulton KJ, Ferreira T, Horikoshi M, Johnson AD, Ng MC, Prokopenko I, Saleheen D, Wang X, Zeggini E, Abecasis GR, Adair LS, Almgren P, Altay M, Aung T, Baldassarre D, Balkau B, Bao Y, Barnett AH, Barroso I, Basit A, Been LF, Beilby J, Bell GI, Benediktsson R, Bergman RN, Boehm BO, Boerwinkle E, Bonnycastle LL, Burtt N, Cai Q, Campbell H, Carey J, Cauchi S, Caulfield M, Chan JC, Chang LC, Chang TJ, Chang YC, Charpentier G, Chen CH, Chen H, Chen YT, Chia KS, Chidambaram M, Chines PS, Cho NH, Cho YM, Chuang LM, Collins FS, Cornelis MC, Couper DJ, Crenshaw AT, van Dam RM, Danesh J, Das D, de Faire U, Dedoussis G, Deloukas P, Dimas AS, Dina C, Doney AS, Donnelly PJ, Dorkhan M, van Duijn C, Dupuis J, Edkins S, Elliott P, Emilsson V, Erbel R, Eriksson JG, Escobedo J, Esko T, Eury E, Florez JC, Fontanillas P, Forouhi NG, Forsen T, Fox C, Fraser RM, Frayling TM, Froguel P, Frossard P, Gao Y, Gertow K, Gieger C, Gigante B, Grallert H, Grant GB, Grop LC, Groves CJ, Grundberg E, Guiducci C, Hamsten A, Han BG, Hara K, Hassanali N, Hattersley AT, Hayward C, Hedman AK, Herder C, Hofman A, Holmen OL, Hovingh K, Hreidarsson AB, Hu C, Hu FB, Hui J, Humphries SE, Hunt SE, Hunter DJ, Hveem K, Hydrie ZI, Ikegami H, Illig T, Ingelsson E, Islam M, Isomaa B, Jackson AU, Jafar T, James A, Jia W, Jöckel KH, Jonsson A, Jowett JB, Kadowaki T, Kang HM, Kanoni S, Kao WH, Kathiresan S, Kato N, Katulanda P, Keinänen-Kiukkaanniemi KM, Kelly AM, Khan H, Khaw KT, Khor CC, Kim HL, Kim S, Kim YJ, Kinnunen L, Klopp N, Kong A, Korpi-Hyövälti E, Kowlessur S, Kraft P, Kravic J, Kristensen MM, Krithika S, Kumar A, Kumate J, Kuusisto J, Kwak SH, Laakso M, Lagou V, Lakka TA, Langenberg C, Langford C, Lawrence R, Leander K, Lee JM, Lee NR, Li M, Li X, Li Y, Liang J, Liju S, Lim WY, Lind L, Lindgren CM, Lindholm E, Liu CT, Liu JJ, Lobbens S, Long J, Loos RJ, Lu W, Luan J, Lyssenko V, Ma RC, Maeda S, Mägi R, Männistö S, Matthews DR, Meigs JB, Melander O, Metspalu A, Meyer J, Mirza G, Mihailov E, Moebus S, Mohan V, Mohlke KL, Morris AD, Mühleisen TW, Müller-Nurasyid M, Musk B, Nakamura J, Nakashima E, Navarro P, Ng PK, Nica AC, Nilsson PM, Njølstad I, Nöthen MM, Ohnaka K, Ong TH, Owen KR, Palmer CN, Pankow JS, Park KS, Parkin M, Pechlivanis S, Pedersen NL, Peltonen L, Perry JR, Peters A, Pinidiyapathirage JM, Platou CG, Potter S, Price JF, Qi L, Radha V, Rallidis L, Rasheed A, Rathman W, Rauramaa R, Raychaudhuri S, Rayner NW, Rees SD, Rehnberg E, Ripatti S, Robertson N, Roden M, Rossin EJ, Rudan I, Rybin D, Saaristo TE, Salomaa V, Saltevo J, Samuel M, Sanghera DK, Saramies J, Scott J, Scott LJ, Scott RA, Segrè AV, Sehmi J, Sennblad B, Shah N, Shah S, Shera AS, Shu XO, Shuldiner AR, Sigurdsson G, Sijbrands E, Silveira A, Sim X, Sivapalaratnam S, Small KS, So WY, Stančáková A, Stefánsson K, Steinbach G, Steinthorsdóttir V, Stirrups K, Strawbridge RJ, Stringham HM, Sun Q, Suo C, Syvänen AC, Takayanagi R, Takeuchi F, Tay WT, Teslovich TM, Thorand B, Thorleifsson G, Thorsteinsdóttir U, Tikkanen E, Trakalo J, Tremoli E, Trip MD, Tsai FJ, Tuomi T, Tuomilehto J, Uitterlinden AG, Valladares-Salgado A, Vedantam S, Veglia F, Voight BF, Wang C, Wareham NJ, Wennauer R, Wickremasinghe AR, Wilsgaard T, Wilson JF, Wiltshire S, Winckler W, Wong TY, Wood AR, Wu JY, Wu Y, Yamamoto K, Yamauchi T, Yang M, Yengo L, Yokota M, Young R, Zabaneh D, Zhang F, Zhang R, Zheng W, Zimmet PZ, Altschuler D, Bowden DW, Cho YS, Cox NJ, Cruz M, Hanis CL, Kooner J, Lee JY, Seielstad M, Teo YY, Boehnke M, Parra EJ, Chambers JC, Tai ES, McCarthy MI, Morris AP. Genome-wide trans-ancestry meta-analysis provides insight into the genetic architecture of type 2 diabetes susceptibility. *Nat Genet* 2014; **46**: 234-244 [PMID: 24509480 DOI: 10.1038/ng.2897]
- 64 **Shah A**, Kanaya AM. Diabetes and associated complications in the South Asian population. *Curr Cardiol Rep* 2014; **16**: 476 [PMID: 24643902 DOI: 10.1007/s11886-014-0476-5]
- 65 **Commodore-Mensah Y**, Samuel LJ, Dennison-Himmelfarb CR, Agyemang C. Hypertension and overweight/obesity in Ghanaians and Nigerians living in West Africa and industrialized countries: a systematic review. *J Hypertens* 2014; **32**: 464-472 [PMID: 24445390 DOI: 10.1097/HJH.000000000000061]
- 66 **Rodriguez F**, Ferdinand KC. Hypertension in minority populations: new guidelines and emerging concepts. *Adv Chronic Kidney Dis* 2015; **22**: 145-153 [PMID: 25704352 DOI: 10.1053/j.ackd.2014.08.004]
- 67 **Razum O**. Commentary: of salmon and time travellers--musing on the mystery of migrant mortality. *Int J Epidemiol* 2006; **35**: 919-921 [PMID: 16847016 DOI: 10.1093/ije/dyl143]
- 68 **Bollini P**, Siem H. No real progress towards equity: health of migrants and ethnic minorities on the eve of the year 2000. *Soc Sci Med* 1995; **41**: 819-828 [PMID: 8571153]
- 69 **Parkin DM**, Khat M. Studies of cancer in migrants: rationale and methodology. *Eur J Cancer* 1996; **32A**: 761-771 [PMID: 9081351]
- 70 **Harding S**, Rosato M, Teyhan A. Trends in cancer mortality among migrants in England and Wales, 1979-2003. *Eur J Cancer* 2009; **45**: 2168-2179 [PMID: 19349162 DOI: 10.1016/j.ejca.2009.02.029]
- 71 **Stirbu I**, Kunst AE, Vlems FA, Visser O, Bos V, Deville W, Nijhuis HG, Coebergh JW. Cancer mortality rates among first and second generation migrants in the Netherlands: Convergence toward the rates of the native Dutch population. *Int J Cancer* 2006; **119**: 2665-2672 [PMID: 16929492 DOI: 10.1002/ijc.22200]
- 72 **Zeeb H**, Razum O, Blettner M, Stegmaier C. Transition in cancer patterns among Turks residing in Germany. *Eur J Cancer* 2002; **38**: 705-711 [PMID: 11916554]
- 73 **Harding S**, Rosato M, Teyhan A. Trends for coronary heart disease and stroke mortality among migrants in England and Wales, 1979-2003: slow declines notable for some groups. *Heart* 2008; **94**: 463-470 [PMID: 17690159 DOI: 10.1136/hrt.2007.122044]
- 74 **Jatrana S**, Pasupuleti SS, Richardson K. Nativity, duration of residence and chronic health conditions in Australia: do trends converge towards the native-born population? *Soc Sci Med* 2014; **119**: 53-63 [PMID: 25150651 DOI: 10.1016/j.socscimed.2014.08.008]
- 75 **Norredam M**, Agyemang C, Hoejbjerg Hansen OK, Petersen JH, Byberg S, Krasnik A, Kunst AE. Duration of residence and disease occurrence among refugees and family reunited immigrants: test of the 'healthy migrant effect' hypothesis. *Trop Med Int Health* 2014; **19**: 958-967 [PMID: 24889930 DOI: 10.1111/tmi.12340]
- 76 **Riosmena F**, Wong R, Palloni A. Migration selection, protection, and acculturation in health: a binational perspective on older adults. *Demography* 2013; **50**: 1039-1064 [PMID: 23192395 DOI: 10.1007/s13524-012-0178-9]
- 77 **Weitoff GR**, Gullberg A, Hjern A, Rosén M. Mortality statistics in immigrant research: method for adjusting underestimation of mortality. *Int J Epidemiol* 1999; **28**: 756-763 [PMID: 10480707]
- 78 **Ramachandran A**, Ma RC, Snehalatha C. Diabetes in Asia. *Lancet* 2010; **375**: 408-418 [PMID: 19875164 DOI: 10.1016/S0140-6736(09)60937-5]
- 79 **Gentilucci UV**, Picardi A, Manfrini S, Khazrai YM, Fioriti E, Altomare M, Guglielmi C, Di Stasio E, Pozzilli P. Westernization of the Filipino population resident in Rome: obesity, diabetes and hypertension. *Diabetes Metab Res Rev* 2008; **24**: 364-370 [PMID: 18273866 DOI: 10.1002/dmrr.807]
- 80 **Bhopal R**, Hayes L, White M, Unwin N, Harland J, Ayis S, Alberti G. Ethnic and socio-economic inequalities in coronary heart disease, diabetes and risk factors in Europeans and South Asians. *J Public Health Med* 2002; **24**: 95-105 [PMID: 12141592]
- 81 **Schouten BC**, Meeuwesen L. Cultural differences in medical communication: a review of the literature. *Patient Educ Couns* 2006; **64**: 21-34 [PMID: 16427760 DOI: 10.1016/j.pec.2005.11.014]

- 82 **Meeuwesen L**, Harmsen JA, Bernsen RM, Bruijnzeels MA. Do Dutch doctors communicate differently with immigrant patients than with Dutch patients? *Soc Sci Med* 2006; **63**: 2407-2417 [PMID: 16928417 DOI: 10.1016/j.socscimed.2006.06.005]
- 83 **Kressin NR**, Wang F, Long J, Bokhour BG, Orner MB, Rothendler J, Clark C, Reddy S, Kozak W, Kroupa LP, Berlowitz DR. Hypertensive patients' race, health beliefs, process of care, and medication adherence. *J Gen Intern Med* 2007; **22**: 768-774 [PMID: 17364243 DOI: 10.1007/s11606-007-0165-9]
- 84 **Andersen RM**. Revisiting the behavioral model and access to medical care: does it matter? *J Health Soc Behav* 1995; **36**: 1-10 [PMID: 7738325]
- 85 **Denktaş S**, Koopmans G, Birnie E, Foets M, Bonsel G. Underutilization of prescribed drugs use among first generation elderly immigrants in the Netherlands. *BMC Health Serv Res* 2010; **10**: 176 [PMID: 20569456 DOI: 10.1186/1472-6963-10-176]
- 86 **Peeters B**, Van Tongelen I, Duran Z, Yüksel G, Mehuys E, Willems S, Remon JP, Boussey K. Understanding medication adherence among patients of Turkish descent with type 2 diabetes: a qualitative study. *Ethn Health* 2015; **20**: 87-105 [PMID: 24588791 DOI: 10.1080/13557858.2014.890174]
- 87 **Mladovsky P**, Ingleby D, McKee M, Rechel B. Good practices in migrant health: the European experience. *Clin Med (Lond)* 2012; **12**: 248-252 [PMID: 22783777]
- 88 **Rechel B**, Mladovsky P, Ingleby D, Mackenbach JP, McKee M. Migration and health in an increasingly diverse Europe. *Lancet* 2013; **381**: 1235-1245 [PMID: 23541058 DOI: 10.1016/S0140-6736(12)62086-8]
- 89 **Fernando E**, Razak F, Lear SA, Anand SS. Cardiovascular Disease in South Asian Migrants. *Can J Cardiol* 2015; **31**: 1139-1150 [PMID: 26321436 DOI: 10.1016/j.cjca.2015.06.008]
- 90 **Dogra S**, Meisner BA, Ardern CI. Variation in mode of physical activity by ethnicity and time since immigration: a cross-sectional analysis. *Int J Behav Nutr Phys Act* 2010; **7**: 75 [PMID: 20946636 DOI: 10.1186/1479-5868-7-75]
- 91 **Sorkin DH**, Biegler KA, Billimek J. Differences in Self-Reported Physical Activity and Body Mass Index Among Older Hispanic and Non-Hispanic White Men and Women: Findings from the 2009 California Health Interview Survey. *J Am Geriatr Soc* 2015; **63**: 2158-2163 [PMID: 26416708 DOI: 10.1111/jgs.13655]
- 92 **Sarich PE**, Ding D, Sitas F, Weber MF. Co-occurrence of chronic disease lifestyle risk factors in middle-aged and older immigrants: A cross-sectional analysis of 264,102 Australians. *Prev Med* 2015; **81**: 209-215 [PMID: 26375966 DOI: 10.1016/j.ypmed.2015.09.004]
- 93 **Fischbacher CM**, Hunt S, Alexander L. How physically active are South Asians in the United Kingdom? A literature review. *J Public Health (Oxf)* 2004; **26**: 250-258 [PMID: 15454592 DOI: 10.1093/pubmed/fdh158]
- 94 **Martin Y**, Collet TH, Bodenmann P, Blum MR, Zimmerli L, Gaspoz JM, Battegay E, Cornuz J, Rodondi N. The lower quality of preventive care among forced migrants in a country with universal healthcare coverage. *Prev Med* 2014; **59**: 19-24 [PMID: 24262974 DOI: 10.1016/j.ypmed.2013.11.006]
- 95 **Ringbäck Weitoft G**, Ericsson O, Löfroth E, Rosén M. Equal access to treatment? Population-based follow-up of drugs dispensed to patients after acute myocardial infarction in Sweden. *Eur J Clin Pharmacol* 2008; **64**: 417-424 [PMID: 18180914 DOI: 10.1007/s00228-007-0425-y]
- 96 **Volodina A**, Bertsche T, Kostev K, Winkler V, Haefeli WE, Becher H. Drug utilization patterns and reported health status in ethnic German migrants (Aussiedler) in Germany: a cross-sectional study. *BMC Public Health* 2011; **11**: 509 [PMID: 21711531 DOI: 10.1186/1471-2458-11-509]
- 97 **Norredam M**, Nielsen SS, Krasnik A. Migrants' utilization of somatic healthcare services in Europe--a systematic review. *Eur J Public Health* 2010; **20**: 555-563 [PMID: 20040522 DOI: 10.1093/eurpub/ckp195]
- 98 **Modesti PA**, Bianchi S, Borghi C, Cameli M, Capasso G, Ceriello A, Ciccone MM, Germanò G, Maiello M, Muiesan ML, Novo S, Padeletti L, Palmiero P, Pillon S, Rotella CM, Saba PS, Scicchitano P, Trimarco B, Volpe M, Pedrinelli R, Di Biase M. Cardiovascular health in migrants: current status and issues for prevention. A collaborative multidisciplinary task force report. *J Cardiovasc Med (Hagerstown)* 2014; **15**: 683-692 [PMID: 25090156 DOI: 10.2459/JCM.0000000000000069]
- 99 **Marchesini G**, Forlani G, Rossi E, Berti A, De Rosa M. The direct economic cost of pharmacologically-treated diabetes in Italy-2006. The ARNO observatory. *Nutr Metab Cardiovasc Dis* 2011; **21**: 339-346 [PMID: 20153612 DOI: 10.1016/j.numecd.2009.10.009]
- 100 **Cadario F**, Cerutti F, Savastio S, Rabbone I, Tumini S, Bruno G. Increasing burden, younger age at onset and worst metabolic control in migrant than in Italian children with type 1 diabetes: an emerging problem in pediatric clinics. *Acta Diabetol* 2014; **51**: 263-267 [PMID: 24065151 DOI: 10.1007/s00592-013-0514-6]
- 101 **Mladovsky P**. Migration and health in the EU, The London School of Economics and Political Science, 2007. Available from: URL: [http://ec.europa.eu/employment\\_social/social\\_situation/docs/rn\\_migration\\_health.pdf](http://ec.europa.eu/employment_social/social_situation/docs/rn_migration_health.pdf)
- 102 **Solé-Auro A**, Guillen M, Crimmins EM. Health care utilization among immigrants and native-born populations in 11 European countries. Results from the Survey of Health, Ageing and Retirement in Europe. 2009/20 WP, Research Institute of Applied Economics, 2009. Available from: URL: [http://www.ub.edu/irea/working\\_papers/2009/200920.pdf](http://www.ub.edu/irea/working_papers/2009/200920.pdf)
- 103 **Imkampe AK**, Gulliford MC. Increasing socio-economic inequality in type 2 diabetes prevalence--repeated cross-sectional surveys in England 1994-2006. *Eur J Public Health* 2011; **21**: 484-490 [PMID: 20685812 DOI: 10.1093/eurpub/ckq106]
- 104 **Tripp-Reimer T**, Choi E, Skemp Kelley L, Enslein JC. Cultural barrier to care: inverting the problem. *Diabetes Spectrum* 2001; **14**: 13-22 [DOI: 10.2337/diaspect.14.1.13]
- 105 **Giorda CB**. The role of the care model in modifying prognosis in diabetes. *Nutr Metab Cardiovasc Dis* 2013; **23**: 11-16 [PMID: 22906566 DOI: 10.1016/j.numecd.2012.07.001]
- 106 **Vlaar EM**, van Valkengoed IG, Nierkens V, Nicolaou M, Middelkoop BJ, Stronks K. Feasibility and effectiveness of a targeted diabetes prevention program for 18 to 60-year-old South Asian migrants: design and methods of the DH!AAN study. *BMC Public Health* 2012; **12**: 371 [PMID: 22621376 DOI: 10.1186/1471-2458-12-371]
- 107 **Hjelm K**, Bard K. Beliefs about health and illness in latin-american migrants with diabetes living in sweden. *Open Nurs J* 2013; **7**: 57-65 [PMID: 23802030 DOI: 10.2174/1874434601307010057]

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## Role of diabetes in heart rhythm disorders

Buelent Koektuerk, Murat Aksoy, Marc Horlitz, Ilkay Bozdog-Turan, Ramazan Goekmen Turan

Buelent Koektuerk, Murat Aksoy, Marc Horlitz, Ramazan Goekmen Turan, Department of Cardiology/Electrophysiology, Witten/Herdecke University, Krankenhaus Porz am Rhein, 58448 Cologne, Germany

Ilkay Bozdog-Turan, Ramazan Goekmen Turan, Division of Cardiology, Department of Internal Medicine, University Hospital, 18055 Rostock, Germany

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**Correspondence to:** Ramazan Goekmen Turan, MD, PhD, Department of Cardiology/Electrophysiology, Witten/Herdecke University, Krankenhaus Porz am Rhein, Alfred-Herrhausen-Straße 50, 58448 Cologne, Germany. [dr\\_g\\_turan@hotmail.com](mailto:dr_g_turan@hotmail.com)  
 Telephone: +49-2203-5666075  
 Fax: +49-2203-5661473

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### Abstract

The incidence of diabetes mellitus (DM) is increasing

rapidly. DM is the leading cause of cardiovascular diseases, which can lead to varied cardiovascular complications by aggravated atherosclerosis in large arteries and coronary atherosclerosis, thereby grows the risk for macro and microangiopathy such as myocardial infarction, stroke, limb loss and retinopathy. Moreover diabetes is one of the strongest and independent risk factor for cardiovascular morbidity and mortality, which associated frequently rhythm disorders such as atrial fibrillation (AF) and ventricular arrhythmias (VA). The present article provides a concise overview of the association between DM and rhythm disorders such as AF and VA with underlying pathophysiological mechanisms.

**Key words:** Atrial fibrillation; Diabetes mellitus; Cardiovascular complications; Pathophysiological mechanisms; Ventricular arrhythmias

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**Core tip:** The incidence of diabetes mellitus (DM) is increasing rapidly. DM is the leading cause of cardiovascular diseases. Atrial fibrillation (AF) and ventricular arrhythmias (VA) are most common form of arrhythmias, which lead to cardiovascular complications and mortality in patients with DM. The present article provides a concise overview of the association between DM and rhythm disorders such as AF and VA with underlying pathophysiological mechanisms.

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### INTRODUCTION

Diabetes mellitus (DM) is recognized as a major cardiovascular (CV) risk factor and its close relationship



with cardiovascular morbidity and mortality is well established<sup>[1]</sup>. Although coronary artery disease and related cardiac events are the most documented diabetic cardiovascular effects, cardiac electrical system is also an important target for diabetic damage. In Framingham heart study, DM is established as an independent risk factor for atrial fibrillation (AF) after 38 years of follow-up<sup>[2]</sup>. A recent meta-analysis published by Huxley *et al*<sup>[3]</sup> revealed that patients with DM had a 40% greater risk of developing AF compared to patients without. On the other hand, there has been growing evidence about the relationship between hypoglycaemic episodes and ventricular rhythm disorders for the recent years<sup>[4,5]</sup>. The relationship between DM and arrhythmic disorders is not fully understood yet and there is a growing population of DM patients everyday. This relationship is expected to become more of an issue in the near future. Here we would like to present a brief overview on this relationship especially for AF and ventricular arrhythmias.

### Diabetes and AF

AF is the most common arrhythmia in clinical practice resulting in major cardiovascular morbidity and mortality<sup>[6]</sup>. Earlier The Framingham Study and recently a study from Movahed *et al*<sup>[7]</sup> clearly established that DM is a powerful and independent risk factor for the development of AF. This close relationship between AF and DM raises the question for pathophysiological basis for this entity. Although there is no single and easy answer for this question, both electrical and anatomical remodelling seems to be important keys of these complex pathophysiological changes.

Extensive fibrosis in the atrial tissue is the anatomical hallmark of AF with a role in both starting and perpetuation of the arrhythmia and as the fibrosis expands it is more likely that paroxysmal AF transforms into permanent or anti arrhythmic resistant type<sup>[8,9]</sup>. Kato *et al*<sup>[10]</sup> showed that DM related atrial fibrosis has a potential role in starting AF in diabetic rat models. Exaggerated systemic and tissue level oxidative stress seems to be the key element in atrial fibrosis related to DM. Dudley *et al*<sup>[11]</sup> evaluated the superoxide anion levels in a pig atrial model. The study compared the levels of superoxide in left atrial appendage between the sinus rhythm group and atrial rapid pacing (ARP) group. Results showed that in the ARP group left atrial superoxide anion levels were almost three times higher than the control group. Another study from Anderson *et al*<sup>[12]</sup> showed that mitochondrial oxidative stress is increased in diabetic human atrial tissue. These studies may show that despite increased levels of systemic oxidative stress in DM, there may be a production of reactive oxygen species *via* mitochondrial pathway specifically at the atrial tissue level.

Non-enzymatic glycosylation of proteins and the end products of this pathway (Advanced Glycation End products; AGEs) interact with their receptors (RAGE) and upregulate the connective tissue growth factor (CTGF)<sup>[13]</sup>. This system (AGERAGE) may start or contribute to atrial fibrosis in diabetic patients *via* stimulation of connective

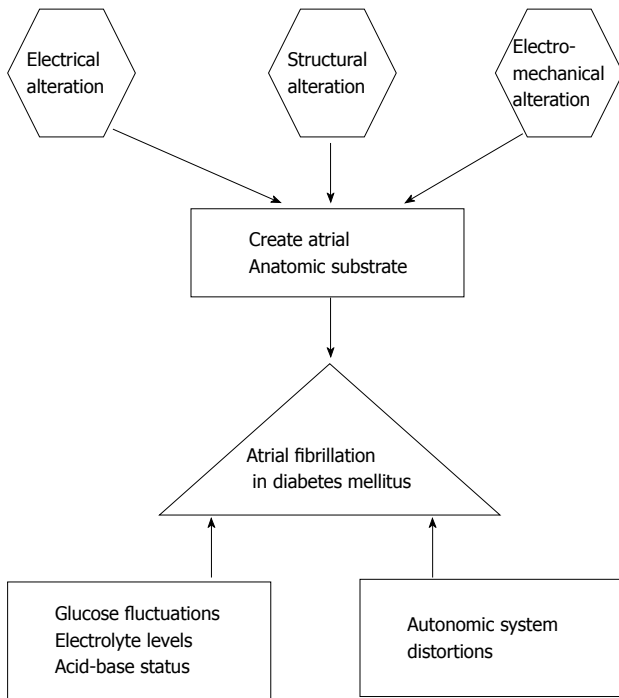
tissue growth factor in the atrial myocardium<sup>[14]</sup>.

Dysfunction in the autonomic innervation and control of cardiovascular system is defined in diabetic patients<sup>[15]</sup>. It seems that autonomic imbalance in favour of sympathetic system, which might have a role in the onset of AF. Otake *et al*<sup>[16]</sup> previously documented that excessive sympathetic stimulation in streptozocin induced diabetic rats increases the incidence of AF compared to control group. Further electrophysiological studies in the diabetic rat atrium revealed shortened atrial effective refractory period (AERP) and increased dispersion of AERP. Another clinical study suggested that reduced heart rate recovery in type 2 DM patients with preserved ejection fraction is associated with increase in the incidence of AF<sup>[17]</sup>. These clinical and laboratory studies imply that autonomic dysfunction has a role in the pathogenesis of AF in DM patients.

Atrial electrical structure is also affected in diabetic patients. Shortened AERP increased dispersion of AERP and intra atrial conduction time, which are the key elements of atrial electrical remodeling<sup>[16,18]</sup>. Chao *et al*<sup>[19]</sup> analyzed the detailed three-dimensional electro anatomic mapping of 228 patients who has DM or abnormal glucose metabolism (AGM) and underwent AF ablation for the first time. Results showed that biatrial voltage measurements in DM and AGM group were significantly lower than control group. Furthermore these patients also had increased recurrence rate of AF in the follow up period. Acar *et al*<sup>[20]</sup> has shown in their published study, that patients with DM had significantly increased inter and intra atrial electro mechanic delay with impaired diastolic functions comparing to control group. On the other hand, it has been shown that there is a specific change in cell to cell integration in DM patients. In a streptozocin induced diabetic rat model study, connexin 43 expression was significantly higher than connexin 40 in diabetic rat atria, which may sign that connexin 43 expression is upregulated in diabetics as a gap junction protein<sup>[21]</sup>.

Conversely, there are some other data suggesting that fluctuations in the blood glucose level rather than the long-term hyperglycemic environment is related to increase in the incidence of AF in diabetic patients<sup>[22]</sup>. Saito *et al*<sup>[23]</sup> showed that glucose fluctuations increase the incidence of AF in streptozocin induced diabetic rat models. Huxley *et al*<sup>[24]</sup> failed to show any correlation between fasting glucose, insulin levels, HbA1c levels measurements and AF onset in patients without diabetes. Another clinical study from Fatemi *et al*<sup>[25]</sup> prospectively evaluated the affect of intense glycemic control on incidence of AF in diabetic patients. Interestingly, they failed to present any association between incident AF and intense therapy comparing to standard therapy group. However, their choice of periodic electrocardiographic testing instead of event recorders might alter the results in terms of missing the paroxysmal AF episodes occurring any time besides the office control.

There are no randomized data specifically addressing the effect of DM in other supraventricular arrhythmias,



**Figure 1** Potential pathophysiological mechanisms of atrial fibrillation in patients with diabetes mellitus.

but there are case reports discussing whether acute changes in metabolic profile during ketoacidosis episodes might trigger arrhythmias such as supraventricular tachycardia<sup>[26]</sup>.

Overall, DM seems to be acting a pivotal role in generation and maintenance of AF in diabetic patients. Specific structural, electrical and electromechanically alterations in diabetic heart might create an anatomic substrate for the development of AF. On the other hand, acute hypo or hyperglycemia changes in electrolyte levels or acid-base status and autonomic system distortions may be a trigger mechanism for the arrhythmia (Figure 1). It is clear that there are still dark spots about the relationship between AF and DM that warrants further studies.

### Diabetes and ventricular arrhythmias

Cardiovascular diseases (CVD) are the leading cause of death in diabetics and DM is almost a synonym for atherosclerosis and coronary artery disease. High incidence and extent of atherosclerotic heart disease in diabetics leads to high incidence of ventricular arrhythmias (VA) and sudden cardiac death (SCD) inevitably<sup>[27-29]</sup>. Although this close relationship between VA, SCDs and DM is mostly based on the extent of coronary artery disease among diabetics, non-coronary atherosclerotic processes like autonomic neuropathy, microvascular disease, ventricular structural and electrical changes may partly play a role in this phenomenon<sup>[30]</sup>.

A ventricular repolarization anomaly, which is reflected by QTc interval prolongation, is associated with high risk of VA. There are several studies showing, marked

QTc prolongation in diabetic patients<sup>[31]</sup>. Another strong predictor of VA, microvolt T wave alternans (TWA) measurement, has been studied in type 2 diabetic patients without known CVD, considering the glycemic status in each patient (which is reflected by HbA1c levels)<sup>[32]</sup>. In this small study, the frequencies of atypical TWA patients were significantly higher than the control group. In the diabetic patient group, patients with atypical TWA measurements had significantly elevated HbA1c levels, which is concluded as every 1% rise in HbA1c levels is linked with 13 fold higher risk of having atypical TWA and suboptimal glycemic control is linked with higher risk of spontaneous VA independent of QTc interval duration. These results from the studies are signing an electrical instability of diabetic myocardium, which creates a potential substrate for ventricular arrhythmias independent from the scarred myocardium areas from previous ischemic cardiac damages. On the other hand, autonomic neuropathy of diabetes results in an unbalanced sympathetic stimulation on myocardium, which may further contribute to this electrical instability and predispose to lethal arrhythmias<sup>[33]</sup>. In this context, cardiac sensory neuropathy of diabetics is another important issue that causes VA and sudden death indirectly *via* silent ischemia<sup>[34]</sup>.

As DM creates a vulnerable myocardium for arrhythmias, it seems to be involved in the triggering mechanism for these arrhythmias too. A study from Chen-Scarabelli *et al.*<sup>[35]</sup> investigated if there is a relationship between HbA1c levels and risk of VA in patients with implantable cardioverter defibrillators retrospectively. The study included 141 patients with DM and 195 patients without DM. A significant association between HbA1c levels of 8%-10% and spontaneous VA incidence in diabetic patients was observed, rather than the diabetic condition and independent from QT prolongation, stating that suboptimal glycemic control and persistent hyperglycemia is related with higher risk of spontaneous VA. On the other hand, in another randomized prospective study, link between hypoglycemic episodes and VA in patients with type 2 DM and documented CVD is investigated<sup>[4]</sup>. In this study, there were 30 patients treated either with insulin and/or sulfonylureas (SU) and there was an age matched control group of 12 patients treated with anti hyperglycemic agents with low risk of hypoglycemia. There were high incidence of hypoglycemia and silent VA in the insulin and/or SU group comparing to control subjects. A study from Pistrosch *et al.*<sup>[5]</sup> further analyzed effects of hypoglycemia as a trigger for VA in larger patient cohort with type 2 DM revealed that hypoglycemia might be able to trigger VA and interestingly a thyroid stimulating hormone level in the low-normal range, which indicates subclinical hyperthyroidism, is independently associated with occurrence of VA. Although these two studies provide no casual relationship between hypoglycemia and VA in diabetic patients, it may be postulated that hypoglycemia may be triggering VA either with sympathetic overstimulation or QT prolongation<sup>[36]</sup>.

Interestingly, another study investigating the association between diabetes and VA in patients with severe

heart failure revealed a negative independent relationship with diabetes and VA in this patient group. Moreover, the authors concluded that DM might have a protective effect for the occurrence of VA in the setting of decompensated heart failure<sup>[37]</sup>.

In summary, DM might play a critical role in creating a vulnerable substrate and/or as a trigger for VA besides the expected risk based on the high extent and incidence of coronary artery disease. Conversely, there is clinical data that claims DM has a protector effect for VA at least in certain group of patients. Unfortunately, there is not enough randomized large-scale data in the literature to suggest a definite relationship and clear pathophysiological mechanisms for this entity.

## CONCLUSION

AF and VA are most common form of arrhythmias, which lead to cardiovascular complications and mortality in patients with DM. Although, there are an evidence based risk factors for an arrhythmogenic substrate that may be specifically related to diabetes, such as heterogeneities in atrial and ventricular repolarization, the extend of myocardial damage, scar formation, autonomic system distortion, glucose fluctuations as well as structural and electrical alterations, the causal pathophysiological and electrophysiological mechanisms are warranted in further studies.

## REFERENCES

- 1 **Garcia MJ**, McNamara PM, Gordon T, Kannel WB. Morbidity and mortality in diabetics in the Framingham population. Sixteen year follow-up study. *Diabetes* 1974; **23**: 105-111 [PMID: 4359625 DOI: 10.2337/diab.23.2.105]
- 2 **Benjamin EJ**, Levy D, Vaziri SM, D'Agostino RB, Belanger AJ, Wolf PA. Independent risk factors for atrial fibrillation in a population-based cohort. The Framingham Heart Study. *JAMA* 1994; **271**: 840-844 [PMID: 8114238 DOI: 10.1001/jama.1994.03510350050036]
- 3 **Huxley RR**, Filion KB, Konety S, Alonso A. Meta-analysis of cohort and case-control studies of type 2 diabetes mellitus and risk of atrial fibrillation. *Am J Cardiol* 2011; **108**: 56-62 [PMID: 21529739]
- 4 **Stahn A**, Pistrosch F, Ganz X, Teige M, Koehler C, Bornstein S, Hanefeld M. Relationship between hypoglycemic episodes and ventricular arrhythmias in patients with type 2 diabetes and cardiovascular diseases: silent hypoglycemia and silent arrhythmias. *Diabetes Care* 2014; **37**: 516-520 [PMID: 24041680 DOI: 10.2337/dc13-0600]
- 5 **Pistrosch F**, Ganz X, Bornstein SR, Birkenfeld AL, Henkel E, Hanefeld M. Risk of and risk factors for hypoglycemia and associated arrhythmias in patients with type 2 diabetes and cardiovascular disease: a cohort study under real-world conditions. *Acta Diabetol* 2015; **52**: 889-895 [PMID: 25749806 DOI: 10.1007/s00592-015-0727-y]
- 6 **Ball J**, Carrington MJ, McMurray JJ, Stewart S. Atrial fibrillation: profile and burden of an evolving epidemic in the 21st century. *Int J Cardiol* 2013; **167**: 1807-1824 [PMID: 23380698 DOI: 10.1016/j.ijcard.2012.12.093]
- 7 **Movahed MR**, Hashemzadeh M, Jamal MM. Diabetes mellitus is a strong, independent risk for atrial fibrillation and flutter in addition to other cardiovascular disease. *Int J Cardiol* 2005; **105**: 315-318 [PMID: 16274775]
- 8 **Burstein B**, Nattel S. Atrial fibrosis: mechanisms and clinical relevance in atrial fibrillation. *J Am Coll Cardiol* 2008; **51**: 802-809 [PMID: 18294563 DOI: 10.1016/j.jacc.2007.09.064]
- 9 **Corradi D**. Atrial fibrillation from the pathologist's perspective. *Cardiovasc Pathol* 2006; **23**: 71-84 [PMID: 24462196 DOI: 10.1016/j.carpath.2013.12.001]
- 10 **Kato T**, Yamashita T, Sekiguchi A, Sagara K, Takamura M, Takata S, Kaneko S, Aizawa T, Fu LT. What are arrhythmogenic substrates in diabetic rat atria? *J Cardiovasc Electrophysiol* 2006; **17**: 890-894 [PMID: 16759295 DOI: 10.1111/j.1540-8167.2006.00528.x]
- 11 **Dudley SC**, Hoch NE, McCann LA, Honeycutt C, Diamandopoulos L, Fukui T, Harrison DG, Dikalov SI, Langberg J. Atrial fibrillation increases production of superoxide by the left atrium and left atrial appendage: role of the NADPH and xanthine oxidases. *Circulation* 2005; **112**: 1266-1273 [PMID: 16129811 DOI: 10.1161/CIRCULATIONAHA.105.538108]
- 12 **Anderson EJ**, Kypson AP, Rodriguez E, Anderson CA, Lehr EJ, Neuffer PD. Substrate-specific derangements in mitochondrial metabolism and redox balance in the atrium of the type 2 diabetic human heart. *J Am Coll Cardiol* 2009; **54**: 1891-1898 [PMID: 19892241 DOI: 10.1016/j.jacc.2009.07.031]
- 13 **Twigg SM**, Cao Z, McLennan SV, Burns WC, Brammar G, Forbes JM, Cooper ME. Renal connective tissue growth factor induction in experimental diabetes is prevented by aminoguanidine. *Endocrinology* 2002; **143**: 4907-4915 [PMID: 12446618 DOI: 10.1210/en.2002-220619]
- 14 **Kato T**, Yamashita T, Sekiguchi A, Tsuneda T, Sagara K, Takamura M, Kaneko S, Aizawa T, Fu LT. AGEs-RAGE system mediates atrial structural remodeling in the diabetic rat. *J Cardiovasc Electrophysiol* 2008; **19**: 415-420 [PMID: 18298515 DOI: 10.1111/j.1540-8167.2007.01037.x]
- 15 **Pop-Busui R**. Cardiac autonomic neuropathy in diabetes: a clinical perspective. *Diabetes Care* 2010; **33**: 434-441 [PMID: 20103559 DOI: 10.2337/dc09-1294]
- 16 **Otake H**, Suzuki H, Honda T, Maruyama Y. Influences of autonomic nervous system on atrial arrhythmogenic substrates and the incidence of atrial fibrillation in diabetic heart. *Int Heart J* 2009; **50**: 627-641 [PMID: 19809211 DOI: 10.1536/ihj.50.627]
- 17 **Negishi K**, Seicean S, Negishi T, Yingchoncharoen T, Aljaroudi W, Marwick TH. Relation of heart-rate recovery to new onset heart failure and atrial fibrillation in patients with diabetes mellitus and preserved ejection fraction. *Am J Cardiol* 2013; **111**: 748-753 [PMID: 23273718 DOI: 10.1016/j.amjcard.2012.11.028]
- 18 **Heijman J**, Voigt N, Nattel S, Dobrev D. Cellular and molecular electrophysiology of atrial fibrillation initiation, maintenance, and progression. *Circ Res* 2014; **114**: 1483-1499 [PMID: 24763466 DOI: 10.1161/CIRCRESAHA.114.302226]
- 19 **Chao TF**, Suenari K, Chang SL, Lin YJ, Lo LW, Hu YF, Tuan TC, Tai CT, Tsao HM, Li CH, Ueng KC, Wu TJ, Chen SA. Atrial substrate properties and outcome of catheter ablation in patients with paroxysmal atrial fibrillation associated with diabetes mellitus or impaired fasting glucose. *Am J Cardiol* 2010; **106**: 1615-1620 [PMID: 21094363 DOI: 10.1016/j.amjcard.2010.07.038]
- 20 **Acar G**, Akcay A, Sokmen A, Ozkaya M, Guler E, Sokmen G, Kaya H, Nacar AB, Tuncer C. Assessment of atrial electromechanical delay, diastolic functions, and left atrial mechanical functions in patients with type 1 diabetes mellitus. *J Am Soc Echocardiogr* 2009; **22**: 732-738 [PMID: 19423291 DOI: 10.1016/j.echo.2009.03.028]
- 21 **Watanabe M**, Yokoshiki H, Mitsuyama H, Mizukami K, Ono T, Tsutsui H. Conduction and refractory disorders in the diabetic atrium. *Am J Physiol Heart Circ Physiol* 2012; **303**: H86-H95 [PMID: 22561303 DOI: 10.1152/ajpheart.00010.2012]
- 22 **Lip GY**, Varughese GI. Diabetes mellitus and atrial fibrillation: perspectives on epidemiological and pathophysiological links. *Int J Cardiol* 2005; **105**: 319-321 [PMID: 16274776 DOI: 10.1016/j.ijcard.2005.03.003]
- 23 **Saito S**, Teshima Y, Fukui A, Kondo H, Nishio S, Nakagawa M, Saikawa T, Takahashi N. Glucose fluctuations increase the incidence of atrial fibrillation in diabetic rats. *Cardiovasc Res* 2014; **104**: 5-14 [PMID: 25082849 DOI: 10.1093/cvr/cvu176]

- 24 **Huxley RR**, Alonso A, Lopez FL, Filion KB, Agarwal SK, Loehr LR, Soliman EZ, Pankow JS, Selvin E. Type 2 diabetes, glucose homeostasis and incident atrial fibrillation: the Atherosclerosis Risk in Communities study. *Heart* 2012; **98**: 133-138 [PMID: 21930722 DOI: 10.1136/heartjnl-2011-300503]
- 25 **Fatemi O**, Yuriditsky E, Tsioufis C, Tsachris D, Morgan T, Basile J, Bigger T, Cushman W, Goff D, Soliman EZ, Thomas A, Papademetriou V. Impact of intensive glycemic control on the incidence of atrial fibrillation and associated cardiovascular outcomes in patients with type 2 diabetes mellitus (from the Action to Control Cardiovascular Risk in Diabetes Study). *Am J Cardiol* 2014; **114**: 1217-1222 [PMID: 25159234 DOI: 10.1016/j.amjcard.2014.07.045]
- 26 **Thomas N**, Scanlon J, Ahmed M. Supraventricular tachycardia in association with diabetic ketoacidosis. *B J Diabetes Vasc Dis* 2007; **7**: 244-245 [DOI: 10.1177/14746514070070050901]
- 27 **Fox CS**, Coady S, Sorlie PD, Levy D, Meigs JB, D'Agostino RB, Wilson PW, Savage PJ. Trends in cardiovascular complications of diabetes. *JAMA* 2004; **292**: 2495-2499 [PMID: 15562129 DOI: 10.1001/jama.292.20.2495]
- 28 **Cho E**, Rimm EB, Stampfer MJ, Willett WC, Hu FB. The impact of diabetes mellitus and prior myocardial infarction on mortality from all causes and from coronary heart disease in men. *J Am Coll Cardiol* 2002; **40**: 954-960 [PMID: 12225722 DOI: 10.1016/S0735-1097(02)02044-2]
- 29 **Balkau B**, Jouven X, Ducimetière P, Eschwege E. Diabetes as a risk factor for sudden death. *Lancet* 1999; **354**: 1968-1969 [PMID: 10622302 DOI: 10.1016/S0140-6736(99)04383-4]
- 30 **Wheeler SG**, Ahroni JH, Boyko EJ. Prospective study of autonomic neuropathy as a predictor of mortality in patients with diabetes. *Diabetes Res Clin Pract* 2002; **58**: 131-138 [PMID: 12213355 DOI: 10.1016/S0168-8227(02)00128-6]
- 31 **Cardoso CR**, Salles GF, Deccache W. Prognostic value of QT interval parameters in type 2 diabetes mellitus: results of a long-term follow-up prospective study. *J Diabetes Complications* 2003; **17**: 169-178 [PMID: 12810239 DOI: 10.1016/S1056-8727(02)00206-4]
- 32 **Molon G**, Costa A, Bertolini L, Zenari L, Arcaro G, Barbieri E, Targher G. Relationship between abnormal microvolt T-wave alternans and poor glycemic control in type 2 diabetic patients. *Pacing Clin Electrophysiol* 2007; **30**: 1267-1272 [PMID: 17897130 DOI: 10.1111/j.1540-8159.2006.00298.x]
- 33 **Vinik AI**, Ziegler D. Diabetic cardiovascular autonomic neuropathy. *Circulation* 2007; **115**: 387-397 [PMID: 17242296 DOI: 10.1161/CIRCULATIONAHA.106.634949]
- 34 **Faerman I**, Faccio E, Milei J, Nuñez R, Jadzinsky M, Fox D, Rapaport M. Autonomic neuropathy and painless myocardial infarction in diabetic patients. Histologic evidence of their relationship. *Diabetes* 1977; **26**: 1147-1158 [PMID: 590638 DOI: 10.2337/diab.26.12.1147]
- 35 **Chen-Scarabelli C**, Scarabelli TM. Suboptimal glycemic control, independently of QT interval duration, is associated with increased risk of ventricular arrhythmias in a high-risk population. *Pacing Clin Electrophysiol* 2006; **29**: 9-14 [PMID: 16441711]
- 36 **Laitinen T**, Lyyra-Laitinen T, Huopio H, Vauhkonen I, Halonen T, Hartikainen J, Niskanen L, Laakso M. Electrocardiographic alterations during hyperinsulinemic hypoglycemia in healthy subjects. *Ann Noninvasive Electrocardiol* 2008; **13**: 97-105 [PMID: 18426434 DOI: 10.1111/j.1542-474X.2008.00208.x]
- 37 **Aronson D**, Burger AJ. Diabetes and the occurrence of ventricular arrhythmic events in patients with severe left ventricular dysfunction. *Diabetologia* 2002; **45**: 1440-1445 [PMID: 12378386 DOI: 10.1007/s00125-002-0915-5]

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Editorial Board Member of *World Journal of Diabetes*, José M Gómez-Sáez, MD, PhD, Research Fellow, Endocrinology Service, Hospital Universitario de Bellvitg, 08907 Barcelona, Spain

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*World Journal of Diabetes*

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Fax: +86-10-85381893

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Baishideng Publishing Group Inc

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## Management of diabolical diabetes mellitus and periodontitis nexus: Are we doing enough?

Abhijit N Gurav

Abhijit N Gurav, Department of Periodontology, Tatyasaheb Kore Dental College and Research Centre, New Pargaon, Kolhapur 416137, Maharashtra State, India

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**Correspondence to:** Abhijit N Gurav, BDS, MDS, Professor, Head of Department of Periodontology, Tatyasaheb Kore Dental College and Research Centre, New Pargaon, NH204 Road, Kolhapur 416137, Maharashtra State, India. [dr\\_abhijitg@yahoo.co.in](mailto:dr_abhijitg@yahoo.co.in)  
Telephone: +91-230-2477081  
Fax: +91-230-2477654

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### Abstract

Periodontitis is the commonest oral disease affecting population worldwide. This disease is notorious for the devastation of tooth supporting structures,

ensuing in the loss of dentition. The etiology for this disease is bacterial biofilm, which accumulates on the teeth as dental plaque. In addition to the biofilm microorganisms, other factors such as environmental, systemic and genetic are also responsible in progression of periodontitis. Diabetes mellitus (DM) is metabolic disorder which has an impact on the global health. DM plays a crucial role in the pathogenesis of periodontitis. Periodontitis is declared as the "sixth" major complication of DM. Evidence based literature has depicted an enhanced incidence and severity of periodontitis in subjects with DM. A "two way" relationship has been purported between periodontitis and DM. Mutual management of both conditions is necessary. Periodontal therapy (PT) may assist to diminish the progression of DM and improve glycemic control. Various advanced technological facilities may be utilized for the purpose of patient education and disease management. The present paper clarifies the etio-pathogenesis of periodontitis, establishing it as a complication of DM and elaborating the various mechanisms involved in the pathogenesis. The role of PT in amelioration of DM and application of digital communication will be discussed. Overall, it is judicious to create an increased patient cognizance of the periodontitis-DM relationship. Conjunctive efforts must be undertaken by the medical and oral health care professionals for the management of periodontitis affected DM patients.

**Key words:** Cost-effectiveness; Advanced glycation end products; Complications; Glycated hemoglobin; Inflammation; Mobile health; Periodontitis; Periodontal therapy; Scaling and root planing; Type 2 diabetes mellitus

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**Core tip:** Various studies have corroborated a two-way relationship between diabetes mellitus and periodontitis. Periodontal therapy (PT) can assist to ameliorate the

glycated hemoglobin levels. Metabolic control in diabetes may prevent further complications. Given the large scale epidemiology of both diabetes and periodontitis, it is prudent for the oral health care personnel to co-ordinate efforts with the diabetes care personnel, for the mutual management of these conditions. The implication of PT in metabolic control of diabetes and the various methods for the systematic management of diabetes-periodontitis nexus are explained in this review.

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## INTRODUCTION

Periodontitis is a common oral disease afflicting humans worldwide, since centuries. Periodontitis is a chronic and painless condition; hence the subject seldom seeks any professional assistance. The lack of treatment results in the destruction of tooth supporting apparatus and further culminates into partial or complete loss of dentition<sup>[1,2]</sup>. Periodontitis is not only a localized condition but also influences systemic health of the individual. This association of periodontitis with systemic health led to the emergence of a novel branch in the field of periodontology, known as periodontal medicine. Periodontal medicine focuses on the aggregation of evidence based data, which establishes a substantial relationship between periodontitis and systemic health<sup>[3]</sup>. Throughout the years periodontitis has been associated with various conditions like diabetes mellitus (DM)<sup>[4]</sup>, cardiovascular atherosclerotic disease<sup>[5]</sup>, preterm low birth weight<sup>[6]</sup>, rheumatoid arthritis<sup>[7]</sup>, cancer<sup>[8]</sup>, chronic kidney disease<sup>[9]</sup>, inflammatory bowel disease<sup>[10]</sup>, obesity<sup>[11]</sup>, metabolic syndrome (MS)<sup>[12]</sup>, dyslipidemia<sup>[13]</sup>, respiratory diseases<sup>[14]</sup>, Alzheimer's disease<sup>[15]</sup> and erectile dysfunction<sup>[16]</sup> and non alcoholic fatty liver disease (NAFLD)<sup>[17]</sup>. It is purported that periodontitis and NAFLD should be included as new components of MS. Both these components harbor a two-way relationship with MS<sup>[18,19]</sup>. Various mechanisms have been proposed for association of various systemic conditions with periodontitis<sup>[20-22]</sup>. The literature is amassed with various studies depicting a relation between periodontitis and DM<sup>[23]</sup>.

DM is a chronic metabolic disorder, involving impaired glucose homeostasis. It is classified as type 1 DM (T1DM) and type 2 DM (T2DM). T1DM is manifested due to the failure of pancreatic  $\beta$  cells to produce sufficient insulin and it is an autoimmune condition. T2DM is due to the resistance offered by peripheral tissues to the action of insulin, regardless of enhanced insulin production by pancreatic  $\beta$  cells. The insulin secretion is augmented in the initial phase of T2DM. This increase in pancreatic

$\beta$  cells function undergoes further deterioration, as in obese individuals. This ensues in the failure of  $\beta$  cells to secrete insulin. This results in hypoinsulinemia with the manifestation of T2DM. Various risk factors are recognized for T2DM. These include genetic susceptibility, pre-existing obesity, smoking, increase in the systemic levels of free fatty acids and pro-inflammatory mediators<sup>[24,25]</sup>. Chronic hyperglycemia, a perennial feature of DM, affects practically all host organs and tissues. The five main cognizable complications of DM include retinopathy, neuropathy, nephropathy, altered wound healing and macrovascular disease. Periodontal disease is added to this list and it is now considered as the "sixth complication" of DM<sup>[26]</sup>.

## METHODS OF DATA COLLECTION

Studies examining the effect of DM on host tissues and periodontal tissues in particular, were identified using PubMed search with key search terms such as "advanced glycation end products", "complications", "cost-effectiveness", "epidemiology", "glycated hemoglobin", "inflammation", "meta-analysis", "mobile health", "oxidative stress", "periodontitis", "periodontal therapy", "telemedicine", "T2DM". Systematic reviews, meta-analysis were also screened. Studies published in English language were considered. The review has been prepared by screening PubMed database from 1992 to April 2015.

## WHAT IS PERIODONTITIS?

Periodontitis is a chronic inflammatory disease of the gingiva. In the United States, the prevalence of periodontal disease is considerably high, affecting almost 47% to 58% of adults<sup>[27]</sup>. The etiology for periodontitis is bacterial plaque which accumulates on the teeth surfaces, in absence of optimal oral hygiene measures. The initial phase of the disease process is called "gingivitis", which clinically presents as swelling and bleeding of gingiva. However, gingivitis is reversible with the resumption of proper oral hygiene. If neglected gingivitis may extend into "periodontitis", which is an irreversible process. Periodontitis results in the gradual deterioration of the periodontium. The periodontium comprises of hard and soft tissue supporting the tooth structure. Clinically, a subject with periodontitis presents accretion of calcified deposits referred to as dental calculus, above (supragingival) and beneath (subgingival) the gingival margin. Edematous, bleeding gingiva, bad breath (halitosis), increase in spacing (diastema) between the teeth, suppuration from periodontal pockets are the other features of periodontitis. Periodontitis is characterized by deepening of the normal gingival sulcus into "periodontal pocket", destruction of supporting fibers and loss of bone. This results in clinical attachment loss, conventionally gauged by an instrument known as periodontal probe (Figure 1). In advanced periodontitis the subject may also reveal loose teeth (referred to as





Figure 1 Deep pockets checked with a periodontal probe in a case of chronic generalized periodontitis.



Figure 2 Radiograph showing bone loss in periodontitis patient.

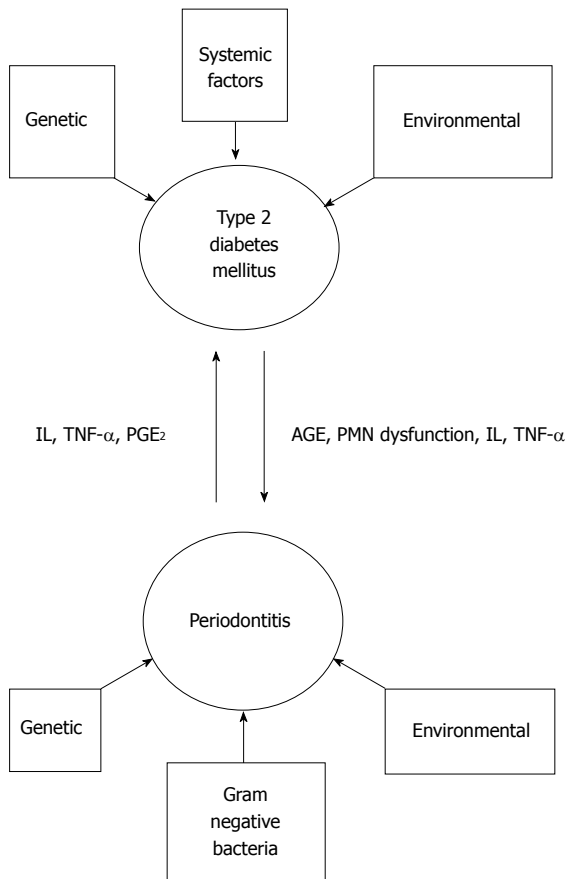
mobile teeth). This condition is evidenced by severe bone loss as observed in the radiograph (Figure 2). The transition of gingivitis to periodontitis is a complex process, involving a qualitative change in microbial biofilm flora. There is an enormous diversity of microbial flora in the dental plaque<sup>[28,29]</sup>. Other factors like local, systemic background and genetic susceptibility also play a crucial role in the advancement of the disease<sup>[30,31]</sup>. Periodontitis is influenced by various modifiable and non modifiable risk factors, which need appraisal<sup>[32]</sup>.

## ETIO-PATHOGENESIS OF PERIODONTITIS AND SYSTEMIC LINK

Periodontitis is a microbial biofilm induced disease. Microbial flora of different strains and species are housed in the biofilm matrix. The transition of gingivitis to periodontitis is subjected to a number of factors including the shift of bacterial species from gram positive aerobic to gram negative anaerobic, host environmental factors and genetics<sup>[33,34]</sup>. Various microbiological molecular techniques have demonstrated the presence of gram negative periodontal pathogens in periodontal disease sites. These include *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Fusobacterium* species, *Treponema denticola*, *Tannerella forsythia*, *Prevotella intermedia*, *Campylobacter rectus*, *Dialister invisus/pneumosintes*<sup>[35]</sup>. With the advances in the field of medicine, the evolution of conceptual models addressing periodontitis has seen many changes. The early concept of the pathogenesis of periodontal disease purported a solo bacterial role in the initiation and progression of periodontitis. However, with ample research and studies, the crucial role of host immune-inflammatory response was highlighted. Eventually, the understanding in the pathogenesis of periodontitis amended with the consideration of microbiologic and immunologic characteristics of the disease. Presently, the most commonly accepted model for the pathogenesis of periodontitis is proposed by Page *et al*<sup>[36]</sup>. It is unanimously accepted by the experts in the domain of periodontal research that bacteria play a

pivotal role in the initiation of the periodontal disease process. However, bacteria are not only adequate for the disease to occur. Host factors such as genetic susceptibility, tobacco and other risk factors play an important role in the severity and ultimate clinical outcome of the periodontal disease. The type of immune response demonstrated by the host periodontal cells on confronting the periodontal pathogen is of crucial significance. This factor determines the resistance or susceptibility of the host tissues to clinical outcome of periodontal disease. Several inflammatory mediators such as interleukin (IL)-1 $\beta$ , prostaglandin (PG) E<sub>2</sub>, tumor necrosis factor (TNF)- $\alpha$  and matrix metalloproteinases (MMPs) orchestrate a significant role in the immuno pathogenesis of periodontal disease<sup>[37]</sup>. The expression of these pro-inflammatory mediators is regulated by the T helper cells. The periodontal bacteria stimulate the innate immune system to produce cytokines, which conduce to the periodontal disease progression<sup>[38]</sup>. The polymorphonuclear neutrophils (PMNs) are important sentinels and serve as the first line of defense in periodontal disease process. However, exaggerated response may transpose this protective action of PMNs into destroyers. Consequently, the periodontium is subjected to damage by an array of mechanisms. PMNs in periodontal disease may at times actually assist in the deterioration of the condition rather than curbing the disease process. Thus, the host immune-inflammatory responses would either be protective or destructive, resembling the proverbial "double edged sword"<sup>[39-41]</sup>. This concept commuted the character of periodontitis to a complex disease, the expression of which implied an obscure interaction between the microbial biofilm and host immune-inflammatory response. This interaction modulates the periodontal tissue homeostasis<sup>[42,43]</sup>.

In a subject with generalized chronic severe periodontitis the total diseased surface area is large. This includes the ulcerated surface of the periodontal pocket epithelium. The total surface area of the periodontal lesions in a severe periodontitis patient is estimated to be 15-20 cm<sup>2</sup><sup>[44]</sup>. Some researchers quantified the periodontal inflamed surface area (PISA) in the range of 0.3 cm<sup>2</sup> in periodontal healthy subjects to 39 cm<sup>2</sup>



**Figure 3** The bidirectional relationship between type 2 diabetes mellitus and periodontitis. IL: Interleukin; TNF- $\alpha$ : Tumor necrosis factor- $\alpha$ ; PGE<sub>2</sub>: Prostaglandin E<sub>2</sub>; AGE: Advanced glycation product; PMN: Polymorphonuclear neutrophil.

in subjects with severe generalized periodontitis. PISA reflects the diseased surface area of the pocket. According to the authors, PISA is an improved version of classification of periodontitis and effectively represents as a risk factor for other diseases<sup>[45]</sup>. The ulcerated pocket surface acts as a portal entry for the periodontal bacteria and various host mediated inflammatory mediators such as IL-1 $\beta$ , TNF- $\alpha$ , PGE<sub>2</sub> and MMPs. The host derived inflammatory mediators along with the bacterial toxic products including lipopolysaccharides are plunged into systemic circulation. Thus, the large surface area of pocket epithelium in periodontitis may pose an inflammatory burden to the body. This shifts the character of periodontitis from an indolent, localized disease to a potential systemic condition. Thus, severe periodontitis is capable of eliciting a low grade systemic inflammation<sup>[38,46]</sup>. A review of the published evidence substantiates modest affiliation between periodontitis and certain systemic diseases<sup>[47]</sup>. However, the relationship between periodontitis and DM is proven to be bidirectional, with both these conditions reciprocating each other (Figure 3). Subjects with DM are at a risk of developing periodontitis and on the other hand periodontitis perturbs the glycemic control in DM subjects<sup>[48]</sup>. The purpose of this review is to highlight the diabolical association of periodontitis and DM. The

measures conducted to reduce the severity of both these chronic, yet debilitating conditions will also be discussed.

## MANAGEMENT OF PERIODONTITIS

The etiology of periodontitis is distinctly associated with dental plaque and calculus deposits, accumulated on the teeth surfaces. Hence, it is prudent to remove these accretions. The initial phase of periodontal therapy (PT) is known as non surgical PT, which consists of scaling and root planing (SRP) of tooth/root surfaces. Periodontal debridement is the "gold standard" in PT<sup>[49]</sup>. SRP involves the mechanical removal of teeth deposits which is accomplished by hand instruments or powered ultrasonic instruments. Curettes and scalers are used for hand instrumentation. The powered ultrasonic scalers facilitate easy removal of calculus deposits. These powered instruments generate a high frequency (range 25000-42000 Hz) vibration of the scaler tip. Water irrigation assists to reduce the frictional heat of the ultrasonic scaler tip<sup>[50-52]</sup>. SRP may fail to eliminate the periodontal pathogens residing in the inaccessible areas like deeper portions of periodontal pockets, concavities on the tooth root and furcation regions of the molar teeth. Systemic antimicrobials serve as useful adjuvants for the treatment of periodontitis. These antimicrobials target the tissue invasive bacteria, not amenable to SRP. These antimicrobials are effective as an adjuvant therapy with SRP and not recommended to be administered as monotherapy<sup>[53,54]</sup>. With the consideration of risk to benefit ratio regarding systemic antimicrobials, the local delivery of antimicrobials proves beneficial. In this local drug delivery (LDD) system, the antimicrobial agents are delivered directly into the periodontal pocket site either in form of fibers, gel, microspheres or chip. The local delivery of antimicrobials counteracts adverse effects related the systemic antimicrobials and conveys a high concentration of the drug at the local periodontal site. These LDD systems act as valuable adjuvant to SRP<sup>[55]</sup>. There are also other adjuvant PT modalities along with SRP. These include photodynamic therapy<sup>[56]</sup> and lasers<sup>[57]</sup>. Lasers have also been used as a substitute or as an adjuvant with mechanical periodontal treatment. In PT laser irradiation has been used for calculus removal and detoxification of diseased root surface, removal of epithelial lining and granulation tissue. Lasers also exert a potential biostimulation effect on the cells in vicinity to the target tissue, thereby abbreviating the inflammation and enhancing the healing of periodontal tissues<sup>[58]</sup>. Host modulation therapy (HMT) may be utilized as an adjunct to SRP. HMT is directed to the manipulation of host inflammatory response. Periodontitis causes host tissue damage *via* the expression of inflammatory mediators such as IL-1, PGE<sub>2</sub>, MMPs. HMT in periodontitis consists of sub antimicrobial dose of antibiotics like doxycycline, non steroidal anti-inflammatory agents like flurbiprofen and bone antiresorptive agents like bisphosphonates<sup>[59]</sup>. Host modulatory agents may be targeted against the expression of host inflammatory mediators and subverting

the inflammatory cell-signalling pathways<sup>[60]</sup>. Surgical therapy can be employed to gain complete access to the diseased periodontal sites and for implementation of regenerative periodontal procedures<sup>[61]</sup>.

## MECHANISMS OF DM INDUCED PERIODONTAL IMPAIRMENT

Epidemiological studies reveal that periodontitis is more prevalent in subjects with DM as compared with the non-diabetic population. DM may increase the risk of periodontitis by two to three folds<sup>[62]</sup>.

DM induced periodontal tissue damage occurs *via* various mechanisms.

These may be categorized as follows: (1) Advanced glycation end products (AGEs) mediated tissue damage; and (2) Immune cell dysfunction.

Oxidative stress induced tissue damage: (1) Chronic hyperglycemia conduces to the formation of AGEs. This occurs as a result of nonenzymatic binding between the reducing sugar moieties with free amine residues of proteins. The AGEs exert their deleterious effects by binding to specific cellular receptor, known as receptor for AGE (RAGE). RAGE plays an important role in the development of DM related complications<sup>[63]</sup>. The AGE-RAGE coupling in DM-periodontitis induced murine models has demonstrated a substantial, sustained inflammatory response. This revealed a progressive bone loss in animal models with DM, compared to those without DM. It was hence deduced that the AGE-RAGE interaction intensifies the destructive process of periodontitis<sup>[64]</sup>. Immune cells such as monocytes, macrophages and PMNs carry a RAGE. AGE provokes these cells to produce excess of superoxide, when challenged by chemoattractants, resulting in tissue destruction<sup>[65,66]</sup>. AGEs are incriminated in exaggerating the periodontal inflammatory responses ensuing in the destruction of periodontal supporting bone<sup>[67]</sup>. The strong and sustained inflammatory response also enhances the process of apoptosis, thus yielding the periodontal tissues to the destructive process of periodontitis. This also diminishes the reparative capacity of the periodontal tissues<sup>[68]</sup>. AGEs combined with pro-inflammatory cytokines can motivate fibroblast apoptosis and impair periodontal wound healing<sup>[69]</sup>. Periodontal tissue collagen cross-linked with AGE shows decreased solubility and high resistance to proteolytic breakdown. This may compromise the physical and mechanical properties of periodontal tissues with greater susceptibility for periodontal disease<sup>[70]</sup>. AGEs also compromise the differentiation, growth and function of osteoblast cells<sup>[71]</sup>; (2) Several studies have analyzed the consequences of chronic hyperglycemia on periodontal tissues. These studies have indicated a dynamic role of exaggerated innate inflammatory response ensuing in microvascular damage, extracellular matrix destruction and ultimately debasement of periodontal tissues<sup>[72-75]</sup>. The PMNs are specialized immune cells which effectuate various

protective and pathological immune responses<sup>[41]</sup>. The PMNs and monocytes are able to express an array of pro-inflammatory cytokines, which regulate the inflammatory process. However, this function is altered in DM. PMNs and monocytes from T2DM subjects expressed greater amount of cytokines as compared to non DM controls<sup>[76,77]</sup>. DM subjects display PMNs with higher vulnerability for oxidative DNA damage as compared to other peripheral blood cells<sup>[78]</sup>. Study on murine animal models has demonstrated that chronic hyperglycemia promotes the increase in the process of PMN margination and macromolecule extravasation in the gingival microvasculature. This may conduce to a pro-inflammatory environment, intensifying the severity of periodontal disease<sup>[79]</sup>. DM activates the innate immune mechanisms and limits the potential for repair. This aggravates the periodontal disease, thus the term "diabetic periodontitis"<sup>[80]</sup>; (3) DM is cognizant to be a state of oxidative stress with exuberant formation of reactive oxygen species (ROS). The increase of ROS in the mitochondrial environment poses a hazard for cellular function. These free radical species permeate through the mitochondrial membrane and gain access to the cellular organelles, resulting in cellular damage. An extensive review of literature by Chapple *et al*<sup>[81]</sup> highlight the far reaching and significant role of ROS in periodontal tissue destruction.

## EFFECT OF DM ON PERIODONTIUM

Histological change in early periodontitis is characterized by the apical migration of the junctional epithelium. Studies conducted on DM rodents have demonstrated the apical recession of the junctional epithelium and inflamed connective tissue. The connective tissue fibers depicted altered arrangement when compared to the non DM controls<sup>[82]</sup>. The gingival epithelium revealed atrophic changes with diminished cellular organelles and increase in the intercellular spaces. DM caused thickening in the keratinized layer and altered differentiation of the epithelial cells<sup>[83]</sup>. Laboratory animal models with DM have shown diminished osteoblasts and periodontal fibroblast cells. A 5 fold increase in osteoblast apoptosis and 2.7 fold increase in periodontal fibroblast apoptosis was observed in these animals<sup>[84]</sup>. Gingival reactive hyperemia was diminished in periodontitis induced rats with DM. The researchers utilized laser flow Doppler flowmetry, to evaluate the attenuated gingival reactive hyperemia. This was attributed to the decrease in vascular endothelial dysfunction in the laboratory animals<sup>[85]</sup>. Gingival tissue in periodontitis human subjects with DM showed an increment in the expression of tissue inhibitors of metalloproteinase (TIMP)-3 and TIMP-4. This expression of TIMP corresponded to the severity of the periodontal disease activity. Increase in TIMP expression is considered as a reciprocal adjustment for the ongoing process of periodontal destruction. The authors have demonstrated a lateral up-regulation



of TIMP in DM subjects with periodontitis<sup>[86]</sup>.

## GLOBAL SCENARIO OF DM AND PERIODONTITIS

The global epidemiology of DM reveals a public health crisis afflicting nearly 382 million people. DM is responsible for the death of almost 5.3 million in 2013. The numbers of people affected with DM keep on escalating and it is proposed that in 2035 it will reach 592 million worldwide. DM is the leading cause of death in South Asian countries and it is menacing the economic status of many countries<sup>[87]</sup>. According to International Diabetes Federation, the South East-Asia region (India, Sri Lanka, Bangladesh, Bhutan, Mauritius, Maldives) inhabits a staggering number of more than 72 million diabetes subjects. Almost 95% of this population constitutes of T2DM<sup>[88]</sup>. China once the capital of DM has been overtaken by India, which is presently the diabetes capital of the world. The etiology of DM in India is multifactorial. This includes genetic and environmental factors, lifestyle of the population. However, it is peculiar to note that in spite of the alarming incidence of DM in India, there are fewer nationwide studies<sup>[89]</sup>. It is estimated that a large number of global population approximately 174.8 million remains to be undiagnosed<sup>[90]</sup>. The global health expenditure is projected to cost 490 billion United States dollars by the year 2030<sup>[91]</sup>. This enormous expenditure on DM is definitely corrosive to the world's greatest economies and poor nations.

Periodontal disease is a global disease afflicting mankind worldwide. Studies reveal that 5% to 20% of any given population is afflicted by severe periodontitis, whereas the mild and moderate forms of periodontitis affect most of the adult population<sup>[92,93]</sup>. The global prevalence of periodontal disease (including gingivitis and periodontitis) is 90%. A British study has reported 8% of the adult population exhibiting a periodontal site with pocket depth  $\geq 6$  mm<sup>[94]</sup>. According to statistical analysis committee on the survey of dental diseases 2005, it is estimated that 70% of Japanese population above the age of 15 years is affected by periodontal disease and 20% of the adult population above 40 years, demonstrate periodontal pocket depth  $\geq 4$  mm<sup>[95]</sup>. The picture in the Indian subcontinent is grave. Studies reveal that 50% of adult population above the age group of 35 years is affected by periodontal disease, ensuing in tooth loss<sup>[96]</sup>. Due to the variation in the global periodontal epidemiological methodology and difference in the case definitions of periodontitis, precise reports concerning periodontal disease prevalence is unavailable<sup>[97]</sup>. The community periodontal index for treatment needs introduced by World Health Organization (WHO) in 1987, is used for epidemiological studies of periodontal disease. This assists various nations to develop a profile of population periodontal status and also effective interventional programs to

tackle periodontal disease related problems<sup>[98]</sup>. According to the 4<sup>th</sup> edition of the WHO manual for oral health surveys, the CPI scores are coded as follows: Score 0: Healthy periodontal status; Score 1: Gingival bleeding; Score 2: Gingival bleeding with presence of calculus; Score 3: Shallow periodontal pocket depth 4-5 mm; Score 4: Deep periodontal pockets  $\geq 6$  mm; Score 9: Excluded; Score X: Not recorded or visible.

The extent of loss of attachment (LA), recorded for a sextant is evaluated by utilizing the following codes: Score 0: LA 0-3 mm; Score 1: LA 4-5 mm; Score 2: LA 6-8 mm; Score 3: LA 9-11 mm; Score 4: LA  $\geq 12$  mm; Score X: Excluded; Score 9: LA not recorded or not visible.

It was observed that severe periodontal disease (with CPI score = 4) is prevalent globally in 10%-15% of adults. The most commonly observed, CPI score of 2 (gingival bleeding and presence of calculus), reflects poor oral hygiene.

The deficiencies of 4<sup>th</sup> edition CPI scoring system have been eliminated with the introduction of the 5<sup>th</sup> edition of the WHO manual for oral health surveys<sup>[93]</sup>. Several leading oral health surveys have used periodontal pocket and attachment loss as the criteria for identifying cases of periodontitis. Presently, epidemiological studies are more concentrated on the appraisal of attachment loss rather than pocket depth<sup>[99,100]</sup>. Workers have expressed mixed reports regarding the prevalence of periodontal disease. The prevalence of periodontal disease was reported to be on a decline in some parts of Europe. However, a surge in prevalence of periodontal disease was noted in Germany and Hungary<sup>[101,102]</sup>. Most of the epidemiological surveys include the population of developed nations. Epidemiological periodontal survey reports from countries like China and India are very limited. These countries harbor a vast population of DM and periodontitis subjects. Methodologies for the application of periodontal epidemiology have seen a steep change. Presently, the conglomeration of data is inadequate to draw tangible conclusions<sup>[92]</sup>.

## EVIDENCE BASED ASSOCIATION OF PERIODONTITIS AND DM

There is a high prevalence of periodontitis in DM subjects. Periodontitis is affirmed to be the sixth complication of DM. The mechanistic link between periodontitis and perturbed glycemic control is still unclear. It is believed that pro-inflammatory mediators such as IL-6 and TNF- $\alpha$  are expressed from the inflamed periodontal sites as a result of microbial stimulus or host response. These mediators enter the systemic circulation and interfere with the function of insulin receptors, thereby deranging the process of insulin signaling. The following cascade of events contributes to insulin resistance and impaired glucose homeostasis<sup>[103]</sup>. Thus, most of the studies carried out with periodontitis subjects and DM have indicated of poor metabolic control. Glucose binds



irreversibly to hemoglobin, leading to the formation of glycosylated hemoglobin (HbA1c). This non-enzymatic glucose moiety persists for the entire life span of the red blood cell. Hence, HbA1c levels reveal the glycemic control of the subject spanning for the previous 1 to 3 mo<sup>[104]</sup>. The recommended HbA1c goal for DM patients is levels < 7%<sup>[105]</sup>.

A large sample size 5-year case-control cohort study was conducted on Japanese subjects, in two groups. The first group consisted of subjects without periodontal pocket depths  $\geq 4$  mm at baseline. These subjects were exposed to an increased risk of developing periodontal pockets of  $\geq 4$  mm after a 5-year period, with HbA1c levels  $\geq 6.5\%$  at baseline. In the other group, subjects with HbA1c < 6.5% and periodontal pockets of 4-5 mm or  $\geq 6$  mm at baseline were likely to display HbA1c levels  $\geq 6.5\%$  after 5 years. This corroborated a direct relationship between periodontal health and glycemic status<sup>[106]</sup>. In a case control study by Wolff *et al*<sup>[107]</sup>, it was concluded that periodontitis is associated with an escalation of HbA1c levels. Thus, periodontitis may skew the glycemic control in subjects without DM, subjecting them to an increased risk of T2DM. The presence of periodontitis in a German study sample without DM, was related to an elevation of HbA1c assay after 5 years of follow-up<sup>[108]</sup>. According to the National Health and Nutrition Examination Survey data, a statistically significant association was noted between HbA1c levels and periodontitis in United States. Subjects with higher HbA1c levels tend to demonstrate more severe periodontal disease<sup>[109]</sup>. A dose-response relationship was observed between PISA and HbA1c, suggesting a causal relationship between periodontitis and T2DM. In T2DM, an increase in PISA results in parallel elevation of the HbA1c levels. It was inferred that with an increase in 333 mm<sup>2</sup> of PISA, the HbA1c level advanced by 1%. Thus, an increase in the periodontal inflammatory burden causes a proportional impairment of glycemic control in T2DM patients<sup>[110]</sup>. PISA can be considered as a predictor of HbA1c along with CRP, in non T2DM subjects. Periodontitis may upset the glycemic control in healthy subjects<sup>[111]</sup>.

## MANAGEMENT OF DIABETES - PERIODONTITIS NEXUS

### Therapeutic methods

Periodontitis compromises the quality of life<sup>[112]</sup>. Both, periodontitis and DM exert a bidirectional influence on each other. Poor glycemic control deteriorates the periodontal health and periodontitis can perturb the glycemic status in a subject with DM. It is noteworthy that the cumulative surface area of the diseased pocket epithelium, in a patient with generalized moderate periodontitis is approximately equal to the surface area of palm of adult hand. In severe periodontitis cases larger surface areas may be involved<sup>[113]</sup>. This is of concern for a health care professional involved in management

of DM, whose primary objective is elimination of all potential foci of infection. It may be certainly anticipated that an infective surface of the magnitude mentioned above can be detrimental for effective glycemic control. Hence, it is prudent to incorporate PT as an important regimen in overall therapeutic management of a subject with DM<sup>[114]</sup>. Competent glycemic control improved periodontal conditions in periodontitis subjects, in absence of any periodontal intervention. This substantiated the "two-way relationship" between DM and periodontitis<sup>[115]</sup>. Various studies have assessed the effect of PT in T2DM patients with periodontitis. These studies included HbA1c as a common parameter to assess the improvement in metabolic control<sup>[116-167]</sup>. These studies have employed SRP alone or combined with systemic/topical antimicrobials. However, there is a variation in the results of these prospective studies. The range in reduction of HbA1c is 0.4% to 2%, with results persisting for 3 to 9 mo after PT. PT ensues in the diminution of serum pro-inflammatory factors such as TNF- $\alpha$  and CRP in T2DM individuals. This accounts for the reduction in systemic inflammatory burden and resultant complications associated with T2DM<sup>[168]</sup>.

Table 1 depicts the various meta-analyses, showing the effect of PT in reduction of HbA1c levels<sup>[169-182]</sup>. Considering the heterogeneity of these studies and limitations in the designs, it would be too premature to draw any explicit conclusions and extrapolations<sup>[170,172]</sup>. To derive robust evidence for implications of PT in DM subjects, further studies should be carried out as per the suggestions furnished by Garcia<sup>[171]</sup>: (1) The trial should be single blind randomized, large sample size, consisting of subjects with DM and moderate/severe periodontitis; (2) The test group should receive the basic PT and the control group should be deferred from any PT; (3) Post PT, the follow-up period should be 6 mo; (4) Results to be evaluated should be based on the parameters assessing periodontal inflammation and metabolic control; and (5) Plasma should be checked for markers of systemic inflammation.

Anti-diabetes drugs are employed as single or combination regimen, for the effective metabolic control in DM patients. The dipeptidyl peptidase-4 inhibitors (known as gliptins) in combination with metformin reduce HbA1c level by 0.8%. When added to metformin and glimepiride, the HbA1c reduction is the same. In comparison to a placebo, gliptins mitigates HbA1c level by 0.6%-0.7%. For DM subjects failing to maintain adequate metabolic control on two anti-diabetic drugs, glucagon-like peptide-1 analogue (exenatide) can be added. Exenatide assists to reduce HbA1c level by about 1%. Pioglitazone, when added to an insulin regimen, favors the HbA1c reduction by about 0.54%<sup>[183]</sup>. The newer sodium-glucose co-transporter 2 inhibitors reduce HbA1c by 0.5%-1.0%<sup>[184]</sup>. It is strategically important to note that a decrement of HbA1c by 1% is associated with a decrease of microvascular complications by 37%, decrease in DM deaths by 21% and a decrease in the risk of myocardial infarction by

**Table 1** Meta-analyses depicting the effect of periodontal therapy on glycated hemoglobin in type 2 diabetes mellitus patients

Ref.	Year	Results	Conclusion
Janket <i>et al</i> <sup>[169]</sup>	2005	7 studies, <i>n</i> = 456	With SRP ↓HbA1c by 0.66% PT and antibiotics ↓HbA1c by 0.71%
Darré <i>et al</i> <sup>[170]</sup>	2008	9 studies, <i>n</i> = 485	PT ↓HbA1c by 0.79%
Garcia <sup>[171]</sup>	2009	9 studies, <i>n</i> = 485	PT ↓HbA1c by 0.79%
Teeuw <i>et al</i> <sup>[172]</sup>	2010	9 studies, <i>n</i> = 371	PT ↓HbA1c by 0.4% in 3 mo
Vergnes <sup>[173]</sup>	2010	7 studies, 3 studies results pooled	SRP (with/without antibiotics) ↓HbA1c by 0.4% in 3-4 mo
Simpson <i>et al</i> <sup>[174]</sup>	2010	7 studies, 3 studies results pooled	SRP (with/without antibiotics) ↓HbA1c by 0.4% in 3-4 mo
Sgolastra <i>et al</i> <sup>[175]</sup>	2013	5 studies	SRP ↓HbA1c by 0.65%
Liew <i>et al</i> <sup>[176]</sup>	2013	6 studies, <i>n</i> = 422	SRP without antibiotics ↓HbA1c by 0.64% in 3 mo
Corbella <i>et al</i> <sup>[177]</sup>	2013	15 studies	PT ↓HbA1c by 0.38% in 3-4 mo and 0.31% in 6 mo
Engelbreton and Kocher <sup>[178]</sup>	2013	9 studies	PT ↓HbA1c by 0.36%
Wang <i>et al</i> <sup>[179]</sup>	2014	10 studies, <i>n</i> = 1135	PT ↓HbA1c by 0.36% in 3 mo and 0.30% in 6 mo
Sun <i>et al</i> <sup>[180]</sup>	2014	8 studies, <i>n</i> = 515	PT ↓HbA1c by 1.03% in 3 mo, 1.17% in 6 mo, 1.21% in 3, 6, 9 mo
Engelbreton <sup>[181]</sup>	2014	7 studies, <i>n</i> = 678	PT ↓HbA1c by 0.38% in 3-4 mo and 0.31% in 6 mo
Li <i>et al</i> <sup>[182]</sup>	2015	9 studies, <i>n</i> = 1082	PT ↓HbA1c by 0.27% in 3 mo

PT: Periodontal treatment; T2DM: Type 2 diabetes mellitus; HbA1c: Glycated hemoglobin; SRP: Scaling and root planing; ↓: Reduced.

14%. Thus, any reduction in HbA1c although miniscule may translate into a decrease in the risk of DM related complications<sup>[185]</sup>.

The anti-diabetic agents are not without adverse effects. Sulfonylureas can cause hypoglycemia. Glucagon like peptide-1 receptor agonists and Metformin exert gastrointestinal side effects<sup>[186-188]</sup>. Thiazolidinediones cause weight gain, peripheral edema and increase the risk of cardiac failure. These agents also elevate the risk of bone fractures, particularly in females<sup>[189,190]</sup>. Sodium-glucose co-transporter 2 inhibitors induce albuminuria, reduce serum uric acid levels, possess diuretic effect and their use is accompanied by genital mycotic infections and urinary tract infections<sup>[191,192]</sup>. An important aspect of anti-diabetic drug therapy is the cost effectiveness. The drug therapy of DM levies a heavy cost, particularly for the developing and populous countries like India and China. Since the costs of newer medications continue to escalate, physicians should exercise their prudent decision regarding different possible options and resources<sup>[183,193,194]</sup>. Theoretically PT may substitute as one or more of the anti-diabetic drugs, employed in the treatment of T2DM. However, more studies should be implemented to substantiate this claim. The practical substitution may reduce the drug related adverse effects and also the cost, in DM patients with periodontitis. Non surgical PT, inclusive of antimicrobial therapy is highly cost effective for the management of periodontitis. This can be effectively used to treat large number of subjects<sup>[195]</sup>.

### Non therapeutic methods

Social support is an important aspect in the management of chronic diseases. Social support can augment the efforts of the health care professionals, ensuing in desirable results<sup>[196]</sup>. Presently, there is a growing body of evidence that peer support significantly assists in amelioration of chronic health conditions. Peer support works on the basis of non stratified reciprocal relationship developed as a result of sharing similar

health ailments<sup>[197]</sup>. A meta-analysis by Qi *et al*<sup>[198]</sup> reveals improvement of HbA1c levels by 0.57% in T2DM patients with peer support approach. In a similar manner, a peer support method may be applied for the management of periodontitis-DM patients. According to an insurance claims data evaluating patients with dental and medical coverage, it was inferred that subjects undergoing PT were salvaged from the medical costs arising as a complication due to DM. This significant reduction measuring 20% to 40% appeared to persist for 3 years<sup>[199]</sup>. Oral diseases may be caused due to altered behavioral pattern. It is imperative to modify the patients' behavioral pattern, adoption of healthy oral care habits coupled with regular visits to the dentists<sup>[200,201]</sup>. As far as the Indian oral health scenario is concerned, the services available to the masses are provided by private and public sectors. Patients with DM are offered state-subsidized public dental care by the municipality. According to an Indian study the public health resources are not fully exploited to the benefit. This study also reported of inadequate oral hygiene in Indians with T2DM and stressed for an enhanced knowledge of periodontal ailments conjugated with oral hygiene methods for disease prevention<sup>[202]</sup>. According to a questionnaire study by Bowyer *et al*<sup>[203]</sup>, it was reported that 69.1% of the participants were not advised by the health care professionals regarding the association between oral hygiene and DM. According to a Jordanian study only 47.7% of the patients with DM were aware of the reciprocal relationship between periodontitis and DM<sup>[204]</sup>. In a German study, 56% of the participants were unaware of the mutual association between periodontitis and DM. It was also noted that 66% of them were not cognizant of the fact that patients with DM are at a greater risk of periodontal disease as compared to the non diabetic counterparts<sup>[205]</sup>. Diabetic patients with periodontitis are often haunted by misconceptions. The most common being discontinuation of brushing and flossing, in order to prevent gums bleed. Patients with DM use alcohol containing

mouthwashes, which actually exacerbates the xerostomia. Thus, misconception and false information about oral hygiene may actually ensue in harmful behaviors. This may set a barricade to an efficient management of periodontal disease in diabetic patients<sup>[206,207]</sup>. Considering the bidirectional relationship between DM and periodontitis it is important to educate, create awareness and motivate patients with DM to maintain scrupulous oral hygiene. Various measures like tooth brushing, flossing and prescribed dental visits are efficacious in maintaining the periodontal health. It is overtly observed that patients with DM are least aware of their periodontal health, incognizant of the fact that poor periodontal health is a cause for perturbed glycemic control. An insignificant number of subjects with DM and periodontitis are aware that PT may assist in the improvement of glycemic status<sup>[208,209]</sup>. Patients with DM keep a routine follow-up with the health care professionals; hence the health care professionals possess a better opportunity to educate these patients about the oral manifestations and complications of DM. They can motivate these patients to dissuade from harmful habits like tobacco. A referral to the oral health professional is important to assess the periodontal status and initiate PT if deemed necessary<sup>[207]</sup>. The oral health professional can also play a crucial role in diagnosis of DM by discerning the typical gingival features in DM. The typical presentation of multiple periodontal abscesses, gingival bleeding and poor response to conventional PT should raise suspicion in the mind of the dentist. Undiagnosed uncontrolled hyperglycemia may be the underlying cause for exaggerated periodontal inflammation<sup>[210]</sup>. A Thai experimental study employed a combination of life-style change and periodontal care in DM patients. The study outcome revealed an improvement in glycemic control and periodontal status of the participants<sup>[211]</sup>. Dentist is traditionally considered specifically as an oral health care professional. Periodontal care in DM subjects is seldom considered by medical authorities. This attitude should undergo a dynamic change, wherein the oral care professional should be incorporated as a strategic member in the team for control of DM<sup>[212]</sup>. The team which disperses proper information about the relationship between DM and periodontal disease should consist of dentists, physicians, diabetes educators. Mass media such as newspaper and television play a key role in everyday life of the masses. The mass media can contribute to betterment in the health of public by the communication of valuable information through educational campaigns, programs and advertisements. The television network in particular has penetrated in all strata of the population constituting an important element. Thus television can sustain positive effects on the health knowledge, attitude and behaviour of the target population. Television programs or advertisements aired for the public health welfare may increase the awareness of the masses about a particular ailment and act as a valuable guide for treatment<sup>[213,214]</sup>. According to a study in Asian subjects, Tokuda *et al*<sup>[215]</sup> concluded that

there exists considerable association between trust in mass media and health of public. This vertical trust of public on the mass media can be effectively exploited for the education of periodontitis-DM association. In today's age of wireless technology a sizeable world population is facilitated by mobile communication. The number of mobile phone subscribers exceeds 6.8 billion worldwide. In India there are 877 million mobile users. The mobile phones in India maintain a low tariff (1.6 United States dollars per month), prompting a large scale utility for communication purposes. This use is popular not only in the urban but also in the rural parts of India. Hence, mobile phones can be exploited as a powerful tool for health care and treatment compliance<sup>[216]</sup>. Health related information and follow-up protocols can be conveyed effectively *via* text messaging<sup>[217,218]</sup>. Text messaging is an effective mode of health education, particularly in rural population lacking computers and internet facility. Moreover, this facility can be utilized by strata of the population possessing basic education<sup>[219]</sup>. Use of mobile in health intervention is particularly encouraging in low and middle income countries. Mobile communication can be used for enhancement of treatment adherence, appointment scheduling, data collection and evolving a network for health care professionals for effectual collaboration<sup>[220,221]</sup>. Patients with medical conditions browse the internet to search for the needful information. The internet is utilized as an effective medium to share clinical information and converse with group of patients with the same ailment. Social networking is an important site to share the needful information. Facebook is one of the most prevalent networking sites with more than 400 million registered users across the globe. Such sites have proved to provide satisfactory support and amend the disease management, to ensue favorable outcomes<sup>[222,223]</sup>. Health care professionals can effectively utilize sophisticated communication devices to spread awareness amongst patients. One such exciting device is the smartphone. Smartphone is widely used by the masses for access of internet, email and data storage, including practicable software application. Management of DM *via* smartphones is an effective tool and holds a bright future<sup>[224,225]</sup>. In South Korea, Kim *et al*<sup>[226]</sup> have reported a smartphone application known as "Diabetes NotePad". This application was designed for the purpose of DM self management, recording of glycemic levels and DM education material. The application can be downloaded free of charge and it is showing increased acceptance and enhanced user satisfaction<sup>[226]</sup>. Gadgets like netbook and smartphone are highly effective for patient counseling, augment patient motivation and self care in management of DM<sup>[227,228]</sup>. Similarly on these lines an application can be developed, addressing the evaluation and recording of periodontal status at every visit. It can also enclose oral hygiene educational material. The smartphone application can remind the patient about the prescribed dental visit and improve communication with the oral health care professional. Application may also be designed to

render effective dissemination of DM-periodontal disease information. With the evolution of periodontal medicine and substantially cognizant periodontal-systemic association, the role of periodontist has evolved from a "gum specialist" to an important collaborator of the medical care team. By the prevention and treatment of periodontal disease periodontists can salvage potential complications arising due to chronic conditions like DM<sup>[229]</sup>.

## CONCLUSION

Oral diseases pose a major challenge to the global public health. It is perceptible that periodontal disease and tooth loss are associated to systemic metabolic disorders such as DM. The past decade has witnessed a plethora of literature unraveling the link between DM and periodontal disease. Various studies have amply elucidated the mechanistic links between the two conditions. It is now discernible that DM has pernicious effects on the periodontal tissues, supporting the tooth. The 2011 New York Academy of Sciences conference on diabetes and oral disease involved the participation of various leading authorities from the fields of medicine and dentistry. The league discussed on the various issues of DM-periodontal disease link and emphasized on the coherent efforts of the medical and oral health professional to achieve optimal outcome of the treatment in DM patients. It is also imperative to counsel the patient and bring home the DM-periodontal disease connection<sup>[230]</sup>. It is hence pertinent to perform epidemiological survey with standard methodologies. A major part of the survey should include population of developing and poor nations. The periodontal and diabetes health care professional community must embrace the technological innovations of information and communication. Mass media, mobiles, smartphones, internet, various social networking sites are all tools for the improved education about the periodontitis-DM and its compound management. Social support also plays a major role in the sustainability of these patients. It is crucial to fortify the public health plans by diagnosis and treatment of chronic oral diseases such as periodontitis. The common risk factors for both these conditions should be identified and necessary approach implemented. The medical professional should comprehend and affirm periodontitis as one of the occult complications of DM and interpolate the oral health professional as an important team member in the treatment of DM.

## REFERENCES

- 1 **Armitage GC**. Periodontal diagnoses and classification of periodontal diseases. *Periodontol 2000* 2004; **34**: 9-21 [PMID: 14717852 DOI: 10.1046/j.09066713.2002.003421.x]
- 2 **Pihlstrom BL**, Michalowicz BS, Johnson NW. Periodontal diseases. *Lancet* 2005; **366**: 1809-1820 [PMID: 16298220 DOI: 10.1016/S01406736(05)67728-8]
- 3 **Williams RC**, Offenbacher S. Periodontal medicine: the emergence of a new branch of periodontology. *Periodontol 2000* 2000; **23**: 9-12

- [PMID: 11276770 DOI: 10.1034/j.1600-0757.2000.2230101.x]
- 4 **Gurav A**, Jadhav V. Periodontitis and risk of diabetes mellitus. *J Diabetes* 2011; **3**: 21-28 [PMID: 20923503 DOI: 10.1111/j.1753-0407.2010.00098.x]
- 5 **Dietrich T**, Sharma P, Walter C, Weston P, Beck J. The epidemiological evidence behind the association between periodontitis and incident atherosclerotic cardiovascular disease. *J Clin Periodontol* 2013; **40** Suppl 14: S70-S84 [PMID: 23627335 DOI: 10.1111/jcpe.12062]
- 6 **Shanthi V**, Vanka A, Bhambal A, Saxena V, Saxena S, Kumar SS. Association of pregnant women periodontal status to preterm and low-birth weight babies: A systematic and evidence-based review. *Dent Res J (Isfahan)* 2012; **9**: 368-380 [PMID: 23162575]
- 7 **Kaur S**, White S, Bartold PM. Periodontal disease and rheumatoid arthritis: a systematic review. *J Dent Res* 2013; **92**: 399-408 [PMID: 23525531 DOI: 10.1177/0022034513483142]
- 8 **Gondivkar SM**, Gondivkar RS, Gadail AR, Chole R, Mankar M, Yuwanati M. Chronic periodontitis and the risk of head and neck squamous cell carcinoma: facts and figures. *Exp Oncol* 2013; **35**: 163-167 [PMID: 24084452]
- 9 **Ruospo M**, Palmer SC, Craig JC, Gentile G, Johnson DW, Ford PJ, Tonelli M, Petrucci M, De Benedittis M, Strippoli GF. Prevalence and severity of oral disease in adults with chronic kidney disease: a systematic review of observational studies. *Nephrol Dial Transplant* 2014; **29**: 364-375 [PMID: 24081863 DOI: 10.1093/ndt/gft401]
- 10 **Vavricka SR**, Manser CN, Hediger S, Vögelin M, Scharl M, Biedermann L, Rogler S, Seibold F, Sanderink R, Attin T, Schoepfer A, Fried M, Rogler G, Frei P. Periodontitis and gingivitis in inflammatory bowel disease: a case-control study. *Inflamm Bowel Dis* 2013; **19**: 2768-2777 [PMID: 24216685]
- 11 **Suvan J**, D'Aiuto F, Moles DR, Petrie A, Donos N. Association between overweight/obesity and periodontitis in adults. A systematic review. *Obes Rev* 2011; **12**: e381-e404 [PMID: 21348914 DOI: 10.1111/j.1467-789X.2010.00808.x]
- 12 **Gurav AN**. The association of periodontitis and metabolic syndrome. *Dent Res J (Isfahan)* 2014; **11**: 1-10 [PMID: 24688553]
- 13 **Lee JB**, Yi HY, Bae KH. The association between periodontitis and dyslipidemia based on the Fourth Korea National Health and Nutrition Examination Survey. *J Clin Periodontol* 2013; **40**: 437-442 [PMID: 23480442 DOI: 10.1111/jcpe.12095]
- 14 **Zeng XT**, Tu ML, Liu DY, Zheng D, Zhang J, Leng W. Periodontal disease and risk of chronic obstructive pulmonary disease: a meta-analysis of observational studies. *PLoS One* 2012; **7**: e46508 [PMID: 23094025 DOI: 10.1371/journal.pone.0046508]
- 15 **Gurav AN**. Alzheimer's disease and periodontitis--an elusive link. *Rev Assoc Med Bras* 2014; **60**: 173-180 [PMID: 24919005 DOI: 10.1590/1806-9282.60.02.015]
- 16 **Oğuz F**, Eltas A, Beytur A, Akdemir E, Uslu MÖ, Güneş A. Is there a relationship between chronic periodontitis and erectile dysfunction? *J Sex Med* 2013; **10**: 838-843 [PMID: 23211042 DOI: 10.1111/j.1743-6109.2012.02974.x]
- 17 **Furuta M**, Ekuni D, Yamamoto T, Irie K, Koyama R, Sanbe T, Yamanaka R, Morita M, Kuroki K, Tobe K. Relationship between periodontitis and hepatic abnormalities in young adults. *Acta Odontol Scand* 2010; **68**: 27-33 [PMID: 19878045 DOI: 10.3109/0016350903291913]
- 18 **Nishimura F**, Soga Y, Iwamoto Y, Kudo C, Murayama Y. Periodontal disease as part of the insulin resistance syndrome in diabetic patients. *J Int Acad Periodontol* 2005; **7**: 16-20 [PMID: 15736891]
- 19 **Tarantino G**, Finelli C. What about non-alcoholic fatty liver disease as a new criterion to define metabolic syndrome? *World J Gastroenterol* 2013; **19**: 3375-3384 [PMID: 23801829 DOI: 10.3748/wjg.v19.i22.3375]
- 20 **Mohangi GU**, Singh-Rambirich S, Volchansky A. Periodontal disease: Mechanisms of infection and inflammation and possible impact on miscellaneous systemic diseases and conditions. *SADJ* 2013; **68**: 462, 464-467 [PMID: 24660421]
- 21 **Linden GJ**, Herzberg MC; Working group 4 of joint EFP/AAP workshop. Periodontitis and systemic diseases: a record of



- discussions of working group 4 of the Joint EFP/AAP Workshop on Periodontitis and Systemic Diseases. *J Clin Periodontol* 2013; **40** Suppl 14: S20-S23 [PMID: 23627330 DOI: 10.1111/jcpe.12091]
- 22 **Otomo-Corgel J**, Pucher JJ, Rethman MP, Reynolds MA. State of the science: chronic periodontitis and systemic health. *J Evid Based Dent Pract* 2012; **12**: 20-28 [PMID: 23040337 DOI: 10.1016/S1532-3382(12)70006-4]
  - 23 **Borgnakke WS**, Ylöstalo PV, Taylor GW, Genco RJ. Effect of periodontal disease on diabetes: systematic review of epidemiologic observational evidence. *J Periodontol* 2013; **84**: S135-S152 [PMID: 23631574 DOI: 10.1902/jop.2013.1340013]
  - 24 **Hu FB**. Globalization of diabetes: the role of diet, lifestyle, and genes. *Diabetes Care* 2011; **34**: 1249-1257 [PMID: 21617109 DOI: 10.2337/dc11-0442]
  - 25 **Gastaldelli A**. Role of beta-cell dysfunction, ectopic fat accumulation and insulin resistance in the pathogenesis of type 2 diabetes mellitus. *Diabetes Res Clin Pract* 2011; **93** Suppl 1: S60-S65 [PMID: 21864753 DOI: 10.1016/S0168-8227(11)70015-8]
  - 26 **Löe H**. Periodontal disease. The sixth complication of diabetes mellitus. *Diabetes Care* 1993; **16**: 329-334 [PMID: 8422804 DOI: 10.2337/diacare.16.1.329]
  - 27 **Eke PI**, Dye BA, Wei L, Thornton-Evans GO, Genco RJ. Prevalence of periodontitis in adults in the United States: 2009 and 2010. *J Dent Res* 2012; **91**: 914-920 [PMID: 22935673 DOI: 10.1177/0022034512457373]
  - 28 **Zijge V**, van Leeuwen MB, Degener JE, Abbas F, Thurnheer T, Gmür R, Harmsen HJ. Oral biofilm architecture on natural teeth. *PLoS One* 2010; **5**: e9321 [PMID: 20195365 DOI: 10.1371/journal.pone.0009321]
  - 29 **Marsh PD**. Dental plaque as a biofilm and a microbial community - implications for health and disease. *BMC Oral Health* 2006; **6** Suppl 1: S14 [PMID: 16934115 DOI: 10.1186/1472-6831-6-S1-S14]
  - 30 **Van Dyke TE**, Sheilesh D. Risk factors for periodontitis. *J Int Acad Periodontol* 2005; **7**: 3-7 [PMID: 15736889]
  - 31 **Heaton B**, Dietrich T. Causal theory and the etiology of periodontal diseases. *Periodontol 2000* 2012; **58**: 26-36 [PMID: 22133365 DOI: 10.1111/j.1600-0757.2011.00414.x]
  - 32 **AlJehani YA**. Risk factors of periodontal disease: review of the literature. *Int J Dent* 2014; **2014**: 182513 [PMID: 24963294 DOI: 10.1155/2014/182513]
  - 33 **Jakubovics NS**, Kolenbrander PE. The road to ruin: the formation of disease-associated oral biofilms. *Oral Dis* 2010; **16**: 729-739 [PMID: 20646235 DOI: 10.1111/j.1601-0825.2010.01701.x]
  - 34 **Marsh PD**, Devine DA. How is the development of dental biofilms influenced by the host? *J Clin Periodontol* 2011; **38** Suppl 11: 28-35 [PMID: 21323701 DOI: 10.1111/j.1600-051X.2010.01673.x]
  - 35 **Armitage GC**. Comparison of the microbiological features of chronic and aggressive periodontitis. *Periodontol 2000* 2010; **53**: 70-88 [PMID: 20403106 DOI: 10.1111/j.1600-0757.2010.00357.x]
  - 36 **Page RC**, Kornman KS. The pathogenesis of human periodontitis: an introduction. *Periodontol 2000* 1997; **14**: 9-11 [PMID: 9567963]
  - 37 **Gemmell E**, Marshall RI, Seymour GJ. Cytokines and prostaglandins in immune homeostasis and tissue destruction in periodontal disease. *Periodontol 2000* 1997; **14**: 112-143 [PMID: 9567968 DOI: 10.1111/j.1600-0757.1997.tb00194.x]
  - 38 **Andruxhov O**, Ulm C, Reischl H, Nguyen PQ, Matejka M, Rausch-Fan X. Serum cytokine levels in periodontitis patients in relation to the bacterial load. *J Periodontol* 2011; **82**: 885-892 [PMID: 21138356 DOI: 10.1902/jop.2010.100425]
  - 39 **Bascones-Martínez A**, Muñoz-Corcuera M, Noronha S, Mota P, Bascones-Ilundain C, Campo-Trapero J. Host defence mechanisms against bacterial aggression in periodontal disease: Basic mechanisms. *Med Oral Patol Oral Cir Bucal* 2009; **14**: e680-e685 [PMID: 19680192]
  - 40 **Nussbaum G**, Shapira L. How has neutrophil research improved our understanding of periodontal pathogenesis? *J Clin Periodontol* 2011; **38** Suppl 11: 49-59 [PMID: 21323704 DOI: 10.1111/j.1600-051X.2010.01678.x]
  - 41 **Kolaczowska E**, Kubes P. Neutrophil recruitment and function in health and inflammation. *Nat Rev Immunol* 2013; **13**: 159-175 [PMID: 23435331 DOI: 10.1038/nri3399]
  - 42 **Kornman KS**. Mapping the pathogenesis of periodontitis: a new look. *J Periodontol* 2008; **79**: 1560-1568 [PMID: 18673011 DOI: 10.1902/jop.2008.080213]
  - 43 **Ishikawa I**. Host responses in periodontal diseases: a preview. *Periodontol 2000* 2007; **43**: 9-13 [PMID: 17214832 DOI: 10.1111/j.1600-0757.2006.00188.x]
  - 44 **Loos BG**. Systemic markers of inflammation in periodontitis. *J Periodontol* 2005; **76**: 2106-2115 [PMID: 16277583]
  - 45 **Nesse W**, Abbas F, van der Ploeg I, Spijkervet FK, Dijkstra PU, Vissink A. Periodontal inflamed surface area: quantifying inflammatory burden. *J Clin Periodontol* 2008; **35**: 668-673 [PMID: 18564145 DOI: 10.1111/j.1600-051X.2008.01249.x]
  - 46 **Ebersole JL**, Stevens J, Steffen MJ, Dawson Iii D, Novak MJ. Systemic endotoxin levels in chronic indolent periodontal infections. *J Periodontol Res* 2010; **45**: 1-7 [PMID: 20465752 DOI: 10.1111/j.1600-0765.2008.01169.x]
  - 47 **Linden GJ**, Lyons A, Scannapieco FA. Periodontal systemic associations: review of the evidence. *J Periodontol* 2013; **84**: S8-S19 [PMID: 23631586 DOI: 10.1902/jop.2013.1340010]
  - 48 **Chapple IL**, Genco R; working group 2 of the joint EFP/AAP workshop. Diabetes and periodontal diseases: consensus report of the Joint EFP/AAP Workshop on Periodontitis and Systemic Diseases. *J Periodontol* 2013; **84**: S106-S112 [PMID: 23631572 DOI: 10.1902/jop.2013.1340011]
  - 49 **Drisko CL**. Periodontal debridement: still the treatment of choice. *J Evid Based Dent Pract* 2014; **14** Suppl: 33-41.e1 [PMID: 24929587 DOI: 10.1016/j.jebdp.2014.02.007]
  - 50 **Tunkel J**, Heinecke A, Flemmig TF. A systematic review of efficacy of machine-driven and manual subgingival debridement in the treatment of chronic periodontitis. *J Clin Periodontol* 2002; **29** Suppl 3: 72-81; discussion 90-91 [PMID: 12787208 DOI: 10.1034/j.1600-051X.29.s3.4.x]
  - 51 **Petersilka GJ**, Ehmke B, Flemmig TF. Antimicrobial effects of mechanical debridement. *Periodontol 2000* 2002; **28**: 56-71 [PMID: 12013348 DOI: 10.1034/j.1600-0757.2002.280103.x]
  - 52 **Oda S**, Nitta H, Setoguchi T, Izumi Y, Ishikawa I. Current concepts and advances in manual and power-driven instrumentation. *Periodontol 2000* 2004; **36**: 45-58 [PMID: 15330943 DOI: 10.1111/j.1600-0757.2004.03674.x]
  - 53 **García Canas P**, Khoully I, Sanz J, Loomer PM. Effectiveness of systemic antimicrobial therapy in combination with scaling and root planing in the treatment of periodontitis: a systematic review. *J Am Dent Assoc* 2015; **146**: 150-163 [PMID: 25726342 DOI: 10.1016/j.adaj.2014.12.015]
  - 54 **Herrera D**, Alonso B, León R, Roldán S, Sanz M. Antimicrobial therapy in periodontitis: the use of systemic antimicrobials against the subgingival biofilm. *J Clin Periodontol* 2008; **35**: 45-66 [PMID: 18724841 DOI: 10.1111/j.1600-051X.2008.01260.x]
  - 55 **Matesanz-Pérez P**, García-Gargallo M, Figuero E, Bascones-Martínez A, Sanz M, Herrera D. A systematic review on the effects of local antimicrobials as adjuncts to subgingival debridement, compared with subgingival debridement alone, in the treatment of chronic periodontitis. *J Clin Periodontol* 2013; **40**: 227-241 [PMID: 23320860 DOI: 10.1111/jcpe.12026]
  - 56 **Sgolastra F**, Petrucci A, Severino M, Graziani F, Gatto R, Monaco A. Adjunctive photodynamic therapy to non-surgical treatment of chronic periodontitis: a systematic review and meta-analysis. *J Clin Periodontol* 2013; **40**: 514-526 [PMID: 23557433 DOI: 10.1111/jcpe.12094]
  - 57 **Aoki A**, Sasaki KM, Watanabe H, Ishikawa I. Lasers in nonsurgical periodontal therapy. *Periodontol 2000* 2004; **36**: 59-97 [PMID: 15330944 DOI: 10.1111/j.1600-0757.2004.03679.x]
  - 58 **Schwarz F**, Aoki A, Becker J, Sculean A. Laser application in non-surgical periodontal therapy: a systematic review. *J Clin Periodontol* 2008; **35**: 29-44 [PMID: 18724840 DOI: 10.1111/j.1600-051X.2008.01259.x]
  - 59 **Kirkwood KL**, Cirelli JA, Rogers JE, Giannobile WV. Novel host response therapeutic approaches to treat periodontal diseases.

- Periodontol* 2000 2007; **43**: 294-315 [PMID: 17214846]
- 60 **Gokhale SR**, Padhye AM. Future prospects of systemic host modulatory agents in periodontal therapy. *Br Dent J* 2013; **214**: 467-471 [PMID: 23660908 DOI: 10.1038/sj.bdj.2013.432]
  - 61 **Heitz-Mayfield LJ**, Lang NP. Surgical and nonsurgical periodontal therapy. Learned and unlearned concepts. *Periodontol* 2000 2013; **62**: 218-231 [PMID: 23574468 DOI: 10.1111/prd.12008]
  - 62 **Mealey BL**, Ocampo GL. Diabetes mellitus and periodontal disease. *Periodontol* 2000 2007; **44**: 127-153 [PMID: 17474930 DOI: 10.1111/j.1600-0757.2006.00193.x]
  - 63 **Gurav AN**. Advanced glycation end products: a link between periodontitis and diabetes mellitus? *Curr Diabetes Rev* 2013; **9**: 355-361 [PMID: 23845077]
  - 64 **Chang PC**, Chien LY, Yeo JF, Wang YP, Chung MC, Chong LY, Kuo MY, Chen CH, Chiang HC, Ng BN, Lee QQ, Phay YK, Ng JR, Erk KY. Progression of periodontal destruction and the roles of advanced glycation end products in experimental diabetes. *J Periodontol* 2013; **84**: 379-388 [PMID: 22554295 DOI: 10.1902/jop.2012.120076]
  - 65 **Festa A**, Schmöler B, Scherthaner G, Menzel EJ. Differential expression of receptors for advanced glycation end products on monocytes in patients with IDDM. *Diabetologia* 1998; **41**: 674-680 [PMID: 9662049 DOI: 10.1007/s001250050967]
  - 66 **Collison KS**, Parhar RS, Saleh SS, Meyer BF, Kwaasi AA, Hammami MM, Schmidt AM, Stern DM, Al-Mohanna FA. RAGE-mediated neutrophil dysfunction is evoked by advanced glycation end products (AGEs). *J Leukoc Biol* 2002; **71**: 433-444 [PMID: 11867681]
  - 67 **Lalla E**, Lamster IB, Stern DM, Schmidt AM. Receptor for advanced glycation end products, inflammation, and accelerated periodontal disease in diabetes: mechanisms and insights into therapeutic modalities. *Ann Periodontol* 2001; **6**: 113-118 [PMID: 11887453 DOI: 10.1902/annals.2001.6.1.113]
  - 68 **Graves DT**, Liu R, Alikhani M, Al-Mashat H, Trackman PC. Diabetes-enhanced inflammation and apoptosis--impact on periodontal pathology. *J Dent Res* 2006; **85**: 15-21 [PMID: 16373675 DOI: 10.1177/154405910608500103]
  - 69 **Desta T**, Li J, Chino T, Graves DT. Altered fibroblast proliferation and apoptosis in diabetic gingival wounds. *J Dent Res* 2010; **89**: 609-614 [PMID: 20354230 DOI: 10.1177/0022034510362960]
  - 70 **Ren L**, Fu Y, Deng Y, Qi L, Jin L. Advanced glycation end products inhibit the expression of collagens type I and III by human gingival fibroblasts. *J Periodontol* 2009; **80**: 1166-1173 [PMID: 19563298 DOI: 10.1902/jop.2009.080669]
  - 71 **Franke S**, Rüster C, Pester J, Hofmann G, Oelzner P, Wolf G. Advanced glycation end products affect growth and function of osteoblasts. *Clin Exp Rheumatol* 2011; **29**: 650-660 [PMID: 21906430]
  - 72 **Gyurko R**, Siqueira CC, Caldon N, Gao L, Kantarci A, Van Dyke TE. Chronic hyperglycemia predisposes to exaggerated inflammatory response and leukocyte dysfunction in Akita mice. *J Immunol* 2006; **177**: 7250-7256 [PMID: 17082643 DOI: 10.4049/jimmunol.177.10.7250]
  - 73 **Taubman MA**, Valverde P, Han X, Kawai T. Immune response: the key to bone resorption in periodontal disease. *J Periodontol* 2005; **76**: 2033-2041 [PMID: 16277573 DOI: 10.1902/jop.2005.76.11-S.2 033]
  - 74 **Amir J**, Waite M, Tobler J, Catalfamo DL, Koutouzis T, Katz J, Waller SM. The role of hyperglycemia in mechanisms of exacerbated inflammatory responses within the oral cavity. *Cell Immunol* 2011; **272**: 45-52 [PMID: 21996642 DOI: 10.1016/j.cellimm.2011.09.008]
  - 75 **Jiang ZL**, Cui YQ, Gao R, Li Y, Fu ZC, Zhang B, Guan CC. Study of TNF- $\alpha$ , IL-1 $\beta$  and LPS levels in the gingival crevicular fluid of a rat model of diabetes mellitus and periodontitis. *Dis Markers* 2013; **34**: 295-304 [PMID: 23478270 DOI: 10.3233/DMA-130974]
  - 76 **Hatanaka A**, Monteagudo PT, Marrocos MS, Campa A. Neutrophils and monocytes as potentially important sources of proinflammatory cytokines in diabetes. *Clin Exp Immunol* 2006; **146**: 443-447 [PMID: 17100763 DOI: 10.1111/j.1365-2249.2006.03229.x]
  - 77 **Salvi GE**, Collins JG, Yalda B, Arnold RR, Lang NP, Offenbacher S. Monocytic TNF alpha secretion patterns in IDDM patients with periodontal diseases. *J Clin Periodontol* 1997; **24**: 8-16 [PMID: 9049792 DOI: 10.1111/j.1600-051X.1997.tb01178.x]
  - 78 **Pitozzi V**, Giovannelli L, Bardini G, Rotella CM, Dolara P. Oxidative DNA damage in peripheral blood cells in type 2 diabetes mellitus: higher vulnerability of polymorphonuclear leukocytes. *Mutat Res* 2003; **529**: 129-133 [PMID: 12943926 DOI: 10.1016/S0027-5107(03)00114-3]
  - 79 **Sima C**, Rhourida K, Van Dyke TE, Gyurko R. Type 1 diabetes predisposes to enhanced gingival leukocyte margination and macromolecule extravasation in vivo. *J Periodontal Res* 2010; **45**: 748-756 [PMID: 20682016 DOI: 10.1111/j.1600-0765.2010.01295.x]
  - 80 **Nassar H**, Kantarci A, van Dyke TE. Diabetic periodontitis: a model for activated innate immunity and impaired resolution of inflammation. *Periodontol* 2000 2007; **43**: 233-244 [PMID: 17214841]
  - 81 **Chapple IL**, Matthews JB. The role of reactive oxygen and antioxidant species in periodontal tissue destruction. *Periodontol* 2000 2007; **43**: 160-232 [PMID: 17214840]
  - 82 **Um YJ**, Jung UW, Kim CS, Bak EJ, Cha JH, Yoo YJ, Choi SH. The influence of diabetes mellitus on periodontal tissues: a pilot study. *J Periodontal Implant Sci* 2010; **40**: 49-55 [PMID: 20498760 DOI: 10.5051/jpis.2010.40.2.49]
  - 83 **Silva JA**, Lorencini M, Reis JR, Carvalho HF, Cagnon VH, Stach-Machado DR. The influence of type I diabetes mellitus in periodontal disease induced changes of the gingival epithelium and connective tissue. *Tissue Cell* 2008; **40**: 283-292 [PMID: 18439638 DOI: 10.1016/j.tice.2008.02.002]
  - 84 **Liu R**, Bal HS, Desta T, Krothapalli N, Alyassi M, Luan Q, Graves DT. Diabetes enhances periodontal bone loss through enhanced resorption and diminished bone formation. *J Dent Res* 2006; **85**: 510-514 [PMID: 16723646]
  - 85 **Sugiyama S**, Takahashi SS, Tokutomi FA, Yoshida A, Kobayashi K, Yoshino F, Wada-Takahashi S, Toyama T, Watanabe K, Hamada N, Todoki K, Lee MC. Gingival vascular functions are altered in type 2 diabetes mellitus model and/or periodontitis model. *J Clin Biochem Nutr* 2012; **51**: 108-113 [PMID: 22962527 DOI: 10.3164/jcbs.11-103]
  - 86 **Jung HY**, Kim YG, Park JW, Suh JY, Lee JM. The expression of a nitric oxide derivative, tissue inhibitors of metalloproteinase-3, and tissue inhibitors of metalloproteinase-4 in chronic periodontitis with type 2 diabetes mellitus. *J Periodontal Implant Sci* 2013; **43**: 87-95 [PMID: 23678392 DOI: 10.5051/jpis.2013.43.2.87]
  - 87 **Rahman MS**, Akter S, Abe SK, Islam MR, Mondal MN, Rahman JA, Rahman MM. Awareness, treatment, and control of diabetes in Bangladesh: a nationwide population-based study. *PLoS One* 2015; **10**: e0118365 [PMID: 25692767 DOI: 10.1371/journal.pone.0118365]
  - 88 **Ramachandran A**, Snehalatha C, Ma RC. Diabetes in South-East Asia: an update. *Diabetes Res Clin Pract* 2014; **103**: 231-237 [PMID: 24300015 DOI: 10.1016/j.diabres.2013.11.011]
  - 89 **Kaveeshwar SA**, Cornwall J. The current state of diabetes mellitus in India. *Australas Med J* 2014; **7**: 45-48 [PMID: 24567766 DOI: 10.4066/AMJ.2013.1979]
  - 90 **Beagley J**, Guariguata L, Weil C, Motala AA. Global estimates of undiagnosed diabetes in adults. *Diabetes Res Clin Pract* 2014; **103**: 150-160 [PMID: 24300018 DOI: 10.1016/j.diabres.2013.11.001]
  - 91 **Zhang P**, Zhang X, Brown J, Vistisen D, Sicree R, Shaw J, Nichols G. Global healthcare expenditure on diabetes for 2010 and 2030. *Diabetes Res Clin Pract* 2010; **87**: 293-301 [PMID: 20171754 DOI: 10.1016/j.diabres.2010.01.026]
  - 92 **Dye BA**. Global periodontal disease epidemiology. *Periodontol* 2000 2012; **58**: 10-25 [PMID: 22133364 DOI: 10.1111/j.1600-0757.2011.00413.x]
  - 93 **Petersen PE**, Ogawa H. The global burden of periodontal disease: towards integration with chronic disease prevention and control. *Periodontol* 2000 2012; **60**: 15-39 [PMID: 22909104 DOI: 10.1111/j.1600-0757.2011.00425.x]

- 94 **Casanova L**, Hughes FJ, Preshaw PM. Diabetes and periodontal disease: a two-way relationship. *Br Dent J* 2014; **217**: 433-437 [PMID: 25342350 DOI: 10.1038/sj.bdj.2014.907]
- 95 **Ueno M**, Takeuchi S, Oshiro A, Shinada K, Ohara S, Kawaguchi Y. Association between diabetes mellitus and oral health status in Japanese adults. *Int J Oral Sci* 2010; **2**: 82-89 [PMID: 20737934 DOI: 10.4248/IJOS10025]
- 96 **Acharya AB**, Satyanarayan A, Thakur SL. Status of association studies linking diabetes mellitus and periodontal disease in India. *Int J Diabetes Dev Ctries* 2010; **30**: 69-74 [PMID: 20535309 DOI: 10.4103/0973-3930.62595]
- 97 **Costa FO**, Guimarães AN, Cota LO, Pataro AL, Segundo TK, Cortelli SC, Costa JE. Impact of different periodontitis case definitions on periodontal research. *J Oral Sci* 2009; **51**: 199-206 [PMID: 19550087]
- 98 **Papapanou PN**. Epidemiology of periodontal diseases: an update. *J Int Acad Periodontol* 1999; **1**: 110-116 [PMID: 12666955]
- 99 **Bourgeois D**, Bouchard P, Mattout C. Epidemiology of periodontal status in dentate adults in France, 2002-2003. *J Periodontol Res* 2007; **42**: 219-227 [PMID: 17451541]
- 100 **Kassebaum NJ**, Bernabé E, Dahiya M, Bhandari B, Murray CJ, Marcenes W. Global burden of severe periodontitis in 1990-2010: a systematic review and meta-regression. *J Dent Res* 2014; **93**: 1045-1053 [PMID: 25261053 DOI: 10.1177/0022034514552491]
- 101 **Sheiham A**, Netuveli GS. Periodontal diseases in Europe. *Periodontol* 2000 2002; **29**: 104-121 [PMID: 12102705]
- 102 **Hugoson A**, Norderyd O. Has the prevalence of periodontitis changed during the last 30 years? *J Clin Periodontol* 2008; **35**: 338-345 [PMID: 18724861 DOI: 10.1111/j.1600-051X.2008.01279.x]
- 103 **Gurav AN**. Periodontitis and insulin resistance: casual or causal relationship? *Diabetes Metab J* 2012; **36**: 404-411 [PMID: 23275933 DOI: 10.4093/dmj.2012.36.6.404]
- 104 **Davidson MB**, Schriger DL, Peters AL, Lorber B. Glycosylated hemoglobin as a diagnostic test for type 2 diabetes mellitus. *JAMA* 2000; **283**: 606-607 [PMID: 10665699]
- 105 **Esposito K**, Chiodini P, Bellastella G, Maiorino MI, Giugliano D. Proportion of patients at HbA1c target & 1%; 7% with eight classes of antidiabetic drugs in type 2 diabetes: systematic review of 218 randomized controlled trials with 78 945 patients. *Diabetes Obes Metab* 2012; **14**: 228-233 [PMID: 21958121 DOI: 10.1111/j.1463-1326.2011.01512.x]
- 106 **Morita I**, Inagaki K, Nakamura F, Noguchi T, Matsubara T, Yoshii S, Nakagaki H, Mizuno K, Sheiham A, Sabbah W. Relationship between periodontal status and levels of glycated hemoglobin. *J Dent Res* 2012; **91**: 161-166 [PMID: 22157098 DOI: 10.1177/0022034511431583]
- 107 **Wolff RE**, Wolff LF, Michalowicz BS. A pilot study of glycosylated hemoglobin levels in periodontitis cases and healthy controls. *J Periodontol* 2009; **80**: 1057-1061 [PMID: 19563284 DOI: 10.1902/jop.2009.080664]
- 108 **Demmer RT**, Desvarieux M, Holtfreter B, Jacobs DR, Wallaschofski H, Nauck M, Völzke H, Kocher T. Periodontal status and A1C change: longitudinal results from the study of health in Pomerania (SHIP). *Diabetes Care* 2010; **33**: 1037-1043 [PMID: 20185742 DOI: 10.2337/dc09-1778]
- 109 **Garcia D**, Tarima S, Okunseri C. Periodontitis and glycemic control in diabetes: NHANES 2009 to 2012. *J Periodontol* 2015; **86**: 499-506 [PMID: 25427615 DOI: 10.1902/jop.2014.140364]
- 110 **Nesse W**, Linde A, Abbas F, Spijkervet FK, Dijkstra PU, de Brabander EC, Gerstenbluth I, Vissink A. Dose-response relationship between periodontal inflamed surface area and HbA1c in type 2 diabetics. *J Clin Periodontol* 2009; **36**: 295-300 [PMID: 19426175 DOI: 10.1111/j.1600-051X.2009.01377.x]
- 111 **Susanto H**, Nesse W, Dijkstra PU, Hoedemaker E, van Reenen YH, Agustina D, Vissink A, Abbas F. Periodontal inflamed surface area and C-reactive protein as predictors of HbA1c: a study in Indonesia. *Clin Oral Invest* 2012; **16**: 1237-1242 [PMID: 22012468 DOI: 10.1007/s00784-011-0621-0]
- 112 **O'Dowd LK**, Durham J, McCracken GI, Preshaw PM. Patients' experiences of the impact of periodontal disease. *J Clin Periodontol* 2010; **37**: 334-339 [PMID: 20447256 DOI: 10.1111/j.1600-051X.2010.01545.x]
- 113 **Page RC**. The pathobiology of periodontal diseases may affect systemic diseases: inversion of a paradigm. *Ann Periodontol* 1998; **3**: 108-120 [PMID: 9722695]
- 114 **Gurav AN**. Periodontal therapy -- an adjuvant for glycemic control. *Diabetes Metab Syndr* 2012; **6**: 218-223 [PMID: 23199544 DOI: 10.1016/j.dsx.2012.09.007]
- 115 **Katagiri S**, Nitta H, Nagasawa T, Izumi Y, Kanazawa M, Matsuo A, Chiba H, Fukui M, Nakamura N, Oseko F, Kanamura N, Inagaki K, Noguchi T, Naruse K, Matsubara T, Miyazaki S, Miyauchi T, Ando Y, Hanada N, Inoue S. Effect of glycemic control on periodontitis in type 2 diabetic patients with periodontal disease. *J Diabetes Invest* 2013; **4**: 320-325 [PMID: 23997922 DOI: 10.1111/jdi.12026]
- 116 **Grossi SG**, Skrepicinski FB, DeCaro T, Robertson DC, Ho AW, Dunford RG, Genco RJ. Treatment of periodontal disease in diabetics reduces glycated hemoglobin. *J Periodontol* 1997; **68**: 713-719 [PMID: 9287060 DOI: 10.1902/jop.1997.68.8.713]
- 117 **Iwamoto Y**, Nishimura F, Nakagawa M, Sugimoto H, Shikata K, Makino H, Fukuda T, Tsuji T, Iwamoto M, Murayama Y. The effect of antimicrobial periodontal treatment on circulating tumor necrosis factor-alpha and glycated hemoglobin level in patients with type 2 diabetes. *J Periodontol* 2001; **72**: 774-778 [PMID: 11453240 DOI: 10.1902/jop.2001.72.6.774]
- 118 **Stewart JE**, Wager KA, Friedlander AH, Zadeh HH. The effect of periodontal treatment on glycemic control in patients with type 2 diabetes mellitus. *J Clin Periodontol* 2001; **28**: 306-310 [PMID: 11314885 DOI: 10.1034/j.1600-051X.2001.028004306.x]
- 119 **Rodrigues DC**, Taba MJ, Novaes AB, Souza SL, Grisi MF. Effect of non-surgical periodontal therapy on glycemic control in patients with type 2 diabetes mellitus. *J Periodontol* 2003; **74**: 1361-1367 [PMID: 14584871 DOI: 10.1902/jop.2003.74.9.1361]
- 120 **Kiran M**, Arpak N, Unsal E, Erdoğan MF. The effect of improved periodontal health on metabolic control in type 2 diabetes mellitus. *J Clin Periodontol* 2005; **32**: 266-272 [PMID: 15766369 DOI: 10.1111/j.1600-051X.2005.00658.x]
- 121 **Promsudthi A**, Pimapsanri S, Deerochanawong C, Kanchanasavita W. The effect of periodontal therapy on uncontrolled type 2 diabetes mellitus in older subjects. *Oral Dis* 2005; **11**: 293-298 [PMID: 16120115 DOI: 10.1111/j.1601-0825.2005.01119.x]
- 122 **Faria-Almeida R**, Navarro A, Bascones A. Clinical and metabolic changes after conventional treatment of type 2 diabetic patients with chronic periodontitis. *J Periodontol* 2006; **77**: 591-598 [PMID: 16584339 DOI: 10.1902/jop.2006.050084]
- 123 **Navarro-Sanchez AB**, Faria-Almeida R, Bascones-Martinez A. Effect of non-surgical periodontal therapy on clinical and immunological response and glycaemic control in type 2 diabetic patients with moderate periodontitis. *J Clin Periodontol* 2007; **34**: 835-843 [PMID: 17850602 DOI: 10.1111/j.1600-051X.2007.01127.x]
- 124 **Jones JA**, Miller DR, Wehler CJ, Rich SE, Krall-Kaye EA, McCoy LC, Christiansen CL, Rothendler JA, Garcia RI. Does periodontal care improve glycemic control? The Department of Veterans Affairs Dental Diabetes Study. *J Clin Periodontol* 2007; **34**: 46-52 [PMID: 17137468 DOI: 10.1111/j.1600-051X]
- 125 **Singh S**, Kumar V, Kumar S, Subbappa A. The effect of periodontal therapy on the improvement of glycemic control in patients with type 2 diabetes mellitus: A randomized controlled clinical trial. *Int J Diabetes Dev Ctries* 2008; **28**: 38-44 [PMID: 19902046 DOI: 10.4103/0973-3930.43097]
- 126 **O'Connell PA**, Taba M, Nomizo A, Foss Freitas MC, Suaid FA, Uyemura SA, Trevisan GL, Novaes AB, Souza SL, Palioto DB, Grisi MF. Effects of periodontal therapy on glycemic control and inflammatory markers. *J Periodontol* 2008; **79**: 774-783 [PMID: 18454655 DOI: 10.1902/jop.2008.070250]
- 127 **Madden TE**, Herriges B, Boyd LD, Laughlin G, Chiodo G, Rosenstein D. Alterations in HbA1c following minimal or enhanced non-surgical, non-antibiotic treatment of gingivitis or mild periodontitis in type 2 diabetic patients: a pilot trial. *J Contemp Dent Pract* 2008; **9**: 9-16 [PMID: 18633464]



- 128 **da Cruz GA**, de Toledo S, Sallum EA, Sallum AW, Ambrosano GM, de Cássia Orlandi Sardi J, da Cruz SE, Gonçalves RB. Clinical and laboratory evaluations of non-surgical periodontal treatment in subjects with diabetes mellitus. *J Periodontol* 2008; **79**: 1150-1157 [PMID: 18597596 DOI: 10.1902/jop.2008.070503]
- 129 **Katagiri S**, Nitta H, Nagasawa T, Uchimura I, Izumiyama H, Inagaki K, Kikuchi T, Noguchi T, Kanazawa M, Matsuo A, Chiba H, Nakamura N, Kanamura N, Inoue S, Ishikawa I, Izumi Y. Multi-center intervention study on glycohemoglobin (HbA1c) and serum, high-sensitivity CRP (hs-CRP) after local anti-infectious periodontal treatment in type 2 diabetic patients with periodontal disease. *Diabetes Res Clin Pract* 2009; **83**: 308-315 [PMID: 19168253 DOI: 10.1016/j.diabetes.2008.10.016]
- 130 **Al-Zahrani MS**, Bamshmous SO, Alhassani AA, Al-Sherbini MM. Short-term effects of photodynamic therapy on periodontal status and glycemic control of patients with diabetes. *J Periodontol* 2009; **80**: 1568-1573 [PMID: 19792844 DOI: 10.1902/jop.2009.090206]
- 131 **Dağ A**, Firat ET, Arian S, Kadiroğlu AK, Kaplan A. The effect of periodontal therapy on serum TNF-alpha and HbA1c levels in type 2 diabetic patients. *Aust Dent J* 2009; **54**: 17-22 [PMID: 19228128 DOI: 10.1111/j.1834-7819.2008.01083.x]
- 132 **Santos VR**, Lima JA, De Mendonça AC, Braz Maximo MB, Faveri M, Duarte PM. Effectiveness of full-mouth and partial-mouth scaling and root planing in treating chronic periodontitis in subjects with type 2 diabetes. *J Periodontol* 2009; **80**: 1237-1245 [PMID: 19656023 DOI: 10.1902/jop.2009.090030]
- 133 **Kudva P**, Tabasum ST, Garg N. Evaluation of clinical and metabolic changes after non surgical periodontal treatment of type 2 diabetes mellitus patients: A clinico biochemical study. *J Indian Soc Periodontol* 2010; **14**: 257-262 [PMID: 21731253 DOI: 10.4103/0972-124X.76933]
- 134 **Kardeşler L**, Buduneli N, Cetinkalp S, Kinane DF. Adipokines and inflammatory mediators after initial periodontal treatment in patients with type 2 diabetes and chronic periodontitis. *J Periodontol* 2010; **81**: 24-33 [PMID: 20059414 DOI: 10.1902/jop.2009.090267]
- 135 **Montoya-Carralero JM**, Saura-Pérez M, Canteras-Jordana M, Morata-Murcia IM. Reduction of HbA1c levels following nonsurgical treatment of periodontal disease in type 2 diabetics. *Med Oral Patol Oral Cir Bucal* 2010; **15**: e808-e812 [PMID: 20383110]
- 136 **Correa FO**, Gonçalves D, Figueredo CM, Bastos AS, Gustafsson A, Orrico SR. Effect of periodontal treatment on metabolic control, systemic inflammation and cytokines in patients with type 2 diabetes. *J Clin Periodontol* 2010; **37**: 53-58 [PMID: 19968741 DOI: 10.1111/j.1600-051X.2009.01498.x]
- 137 **Sun WL**, Chen LL, Zhang SZ, Wu YM, Ren YZ, Qin GM. Inflammatory cytokines, adiponectin, insulin resistance and metabolic control after periodontal intervention in patients with type 2 diabetes and chronic periodontitis. *Intern Med* 2011; **50**: 1569-1574 [PMID: 21804283]
- 138 **Koromantzios PA**, Makrilakis K, Dereka X, Katsilambros N, Vrotsos IA, Madianos PN. A randomized, controlled trial on the effect of non-surgical periodontal therapy in patients with type 2 diabetes. Part I: effect on periodontal status and glycaemic control. *J Clin Periodontol* 2011; **38**: 142-147 [PMID: 21114680 DOI: 10.1111/j.1600-051X.2010.01652.x]
- 139 **Calabrese N**, D'Aiuto F, Calabrese A, Patel K, Calabrese G, Massi-Benedetti M. Effects of periodontal therapy on glucose management in people with diabetes mellitus. *Diabetes Metab* 2011; **37**: 456-459 [PMID: 21757386 DOI: 10.1016/j.diabet.2011.05.004]
- 140 **Engbreton SP**, Hey-Hadavi J. Sub-antimicrobial doxycycline for periodontitis reduces hemoglobin A1c in subjects with type 2 diabetes: a pilot study. *Pharmacol Res* 2011; **64**: 624-629 [PMID: 21782948 DOI: 10.1016/j.phrs.2011.06.024]
- 141 **Hungund S**, Panseriya BJ. Reduction in HbA1c levels following non-surgical periodontal therapy in type-2 diabetic patients with chronic generalized periodontitis: A periodontist's role. *J Indian Soc Periodontol* 2012; **16**: 16-21 [PMID: 22628957 DOI: 10.4103/0972-124X.94598]
- 142 **Koromantzios PA**, Makrilakis K, Dereka X, Offenbacher S, Katsilambros N, Vrotsos IA, Madianos PN. Effect of non-surgical periodontal therapy on C-reactive protein, oxidative stress, and matrix metalloproteinase (MMP)-9 and MMP-2 levels in patients with type 2 diabetes: a randomized controlled study. *J Periodontol* 2012; **83**: 3-10 [PMID: 21627458 DOI: 10.1902/jop.2011.110148]
- 143 **Serrano C**, Pérez C, Sabogal D. Effect of periodontal therapy on metabolic control and an inflammatory mediator in type 2 diabetic subjects: a report on 17 consecutive cases. *J Int Acad Periodontol* 2012; **14**: 26-34 [PMID: 22799126]
- 144 **Dodwad V**, Ahuja S, Kukreja BJ. Effect of locally delivered tetracycline hydrochloride as an adjunct to scaling and root planing on HbA1c, C-reactive protein, and lipid profile in type 2 diabetes: A clinico-biochemical study. *Contemp Clin Dent* 2012; **3**: 150-154 [PMID: 22919212 DOI: 10.4103/0976-237X.96816]
- 145 **Moeintaghavi A**, Arab HR, Bozorgnia Y, Kianoush K, Alizadeh M. Non-surgical periodontal therapy affects metabolic control in diabetics: a randomized controlled clinical trial. *Aust Dent J* 2012; **57**: 31-37 [PMID: 22369555 DOI: 10.1111/j.1834-7819.2011.01652.x]
- 146 **Lin SJ**, Tu YK, Tsai SC, Lai SM, Lu HK. Non-surgical periodontal therapy with and without subgingival minocycline administration in patients with poorly controlled type II diabetes: a randomized controlled clinical trial. *Clin Oral Investig* 2012; **16**: 599-609 [PMID: 21416238 DOI: 10.1007/s00784-011-0535-x]
- 147 **Chen L**, Luo G, Xuan D, Wei B, Liu F, Li J, Zhang J. Effects of non-surgical periodontal treatment on clinical response, serum inflammatory parameters, and metabolic control in patients with type 2 diabetes: a randomized study. *J Periodontol* 2012; **83**: 435-443 [PMID: 21859323 DOI: 10.1902/jop.2011.110327]
- 148 **Auyeung L**, Wang PW, Lin RT, Hsieh CJ, Lee PY, Zhuang RY, Chang HW. Evaluation of periodontal status and effectiveness of non-surgical treatment in patients with type 2 diabetes mellitus in Taiwan for a 1-year period. *J Periodontol* 2012; **83**: 621-628 [PMID: 21692625 DOI: 10.1902/jop.2011.110133]
- 149 **Katagiri S**, Nagasawa T, Kobayashi H, Takamatsu H, Bharti P, Izumiyama H, Uchimura I, Tagami T, Suzuki T, Nanbara H, Taniguchi Y, Hayakumo S, Koyanagi T, Himeno-Ando A, Goto M, Kajio H, Takahashi Y, Izumi Y, Noda M. Improvement of glycemic control after periodontal treatment by resolving gingival inflammation in type 2 diabetic patients with periodontal disease. *J Diabetes Investig* 2012; **3**: 402-409 [PMID: 24843597 DOI: 10.1111/j.2040-1124.2012.00209.x]
- 150 **Cirano FR**, Pera C, Ueda P, Casarin RC, Ribeiro FV, Pimentel SP, Casati MZ. Clinical and metabolic evaluation of one-stage, full-mouth, ultrasonic debridement as a therapeutic approach for uncontrolled type 2 diabetic patients with periodontitis. *Quintessence Int* 2012; **43**: 671-681 [PMID: 23034420]
- 151 **Santos VR**, Lima JA, Miranda TS, Gonçalves TE, Figueiredo LC, Faveri M, Duarte PM. Full-mouth disinfection as a therapeutic protocol for type-2 diabetic subjects with chronic periodontitis: twelve-month clinical outcomes: a randomized controlled clinical trial. *J Clin Periodontol* 2013; **40**: 155-162 [PMID: 23305133 DOI: 10.1111/jcpe.12040]
- 152 **Camargo GA**, Lima Mde A, Fortes TV, de Souza CS, de Jesus AM, de Almeida RP. Effect of periodontal therapy on metabolic control and levels of IL-6 in the gingival crevicular fluid in type 2 diabetes mellitus. *Indian J Dent Res* 2013; **24**: 110-116 [PMID: 23852243 DOI: 10.4103/0970-9290.114953]
- 153 **Bharti P**, Katagiri S, Nitta H, Nagasawa T, Kobayashi H, Takeuchi Y, Izumiyama H, Uchimura I, Inoue S, Izumi Y. Periodontal treatment with topical antibiotics improves glycemic control in association with elevated serum adiponectin in patients with type 2 diabetes mellitus. *Obes Res Clin Pract* 2013; **7**: e129-e138 [PMID: 24331774 DOI: 10.1016/j.orcp.2011.11.005]
- 154 **Munenaga Y**, Hiroshima Study Group, Yamashina T, Tanaka J, Nishimura F. Improvement of glycated hemoglobin in Japanese subjects with type 2 diabetes by resolution of periodontal inflammation using adjunct topical antibiotics: results from the Hiroshima Study. *Diabetes Res Clin Pract* 2013; **100**: 53-60 [PMID: 23465365 DOI: 10.1016/j.diabetes.2013.01.028]



- 155 **Engelbreton SP**, Hyman LG, Michalowicz BS, Schoenfeld ER, Gelato MC, Hou W, Seaquist ER, Reddy MS, Lewis CE, Oates TW, Tripathy D, Katancik JA, Orlander PR, Paquette DW, Hanson NQ, Tsai MY. The effect of nonsurgical periodontal therapy on hemoglobin A1c levels in persons with type 2 diabetes and chronic periodontitis: a randomized clinical trial. *JAMA* 2013; **310**: 2523-2532 [PMID: 24346989 DOI: 10.1001/jama.2013.282431]
- 156 **Gaikwad SP**, Gurav AN, Shete AR, Desarda HM. Effect of scaling and root planing combined with systemic doxycycline therapy on glycemic control in diabetes mellitus subjects with chronic generalized periodontitis: a clinical study. *J Periodontal Implant Sci* 2013; **43**: 79-86 [PMID: 23678391 DOI: 10.5051/jpis.2013.43.2.79]
- 157 **Telgi RL**, Tandon V, Tangade PS, Tirth A, Kumar S, Yadav V. Efficacy of nonsurgical periodontal therapy on glycaemic control in type II diabetic patients: a randomized controlled clinical trial. *J Periodontal Implant Sci* 2013; **43**: 177-182 [PMID: 24040570 DOI: 10.5051/jpis.2013.43.4.177]
- 158 **DPTT study group**, Engelbreton S, Gelato M, Hyman L, Michalowicz BS, Schoenfeld E. Design features of the Diabetes and Periodontal Therapy Trial (DPTT): a multicenter randomized single-masked clinical trial testing the effect of nonsurgical periodontal therapy on glycosylated hemoglobin (HbA1c) levels in subjects with type 2 diabetes and chronic periodontitis. *Contemp Clin Trials* 2013; **36**: 515-526 [PMID: 24080100 DOI: 10.1016/j.cct.2013.09.010]
- 159 **Botero JE**, Yepes FL, Ochoa SP, Hincapie JP, Roldan N, Ospina CA, Castrillon CA, Becerra MA. Effects of periodontal non-surgical therapy plus azithromycin on glycemic control in patients with diabetes: a randomized clinical trial. *J Periodontal Res* 2013; **48**: 706-712 [PMID: 23441920 DOI: 10.1111/jre.12058]
- 160 **Macedo Gde O**, Novaes AB, Souza SL, Taba M, Palioto DB, Grisi MF. Additional effects of aPDT on nonsurgical periodontal treatment with doxycycline in type II diabetes: a randomized, controlled clinical trial. *Lasers Med Sci* 2014; **29**: 881-886 [PMID: 23474741 DOI: 10.1007/s10103-013-1285-6]
- 161 **Raman RP**, Taiyeb-Ali TB, Chan SP, Chinna K, Vaithilingam RD. Effect of nonsurgical periodontal therapy versus oral hygiene instructions on type 2 diabetes subjects with chronic periodontitis: a randomised clinical trial. *BMC Oral Health* 2014; **14**: 79 [PMID: 24965218 DOI: 10.1186/1472-6831-14-79]
- 162 **Gay IC**, Tran DT, Cavender AC, Weltman R, Chang J, Luckenbach E, Tribble GD. The effect of periodontal therapy on glycaemic control in a Hispanic population with type 2 diabetes: a randomized controlled trial. *J Clin Periodontol* 2014; **41**: 673-680 [PMID: 24797222 DOI: 10.1111/jcpe.12268]
- 163 **Soorya KV**, Suchetha A, Lakshmi P, Sapna N, Apoorva SM, Bhat D, Mundinamane DB. The Effect of Scaling and Root Planing on Glycaemic Control, Periodontal Status and Gingival Crevicular Fluid TNF- $\alpha$  Levels in an Indian Population- To Reveal the Ambivalent Link. *J Clin Diagn Res* 2014; **8**: ZC22-ZC26 [PMID: 25584310 DOI: 10.7860/JCDR/2014/9490.5115]
- 164 **Michalowicz BS**, Hyman L, Hou W, Oates TW, Reddy M, Paquette DW, Katancik JA, Engelbreton SP; Diabetes and Periodontal Therapy Trial Study Team. Factors associated with the clinical response to nonsurgical periodontal therapy in people with type 2 diabetes mellitus. *J Am Dent Assoc* 2014; **145**: 1227-1239 [PMID: 25429036 DOI: 10.14219/jada.2014.92]
- 165 **Koromantzios PA**, Madianos P. Nonsurgical periodontal treatment can improve HbA1c values in a Mexican-American population of patients with type 2 diabetes mellitus (DM2) and periodontal disease (PD). *J Evid Based Dent Pract* 2014; **14**: 193-194 [PMID: 25488871 DOI: 10.1016/j.jebdp.2014.10.005]
- 166 **Acharya AB**, Thakur S, Muddapur MV. Effect of scaling and root planing on serum interleukin-10 levels and glycemic control in chronic periodontitis and type 2 diabetes mellitus. *J Indian Soc Periodontol* 2015; **19**: 188-193 [PMID: 26015670 DOI: 10.4103/0972-124X.148644]
- 167 **Wu Y**, Chen L, Wei B, Luo K, Yan F. Effect of non-surgical periodontal treatment on visfatin concentrations in serum and gingival crevicular fluid of patients with chronic periodontitis and type 2 diabetes mellitus. *J Periodontol* 2015; **86**: 795-800 [PMID: 25786566 DOI: 10.1902/jop.2015.140476]
- 168 **Artese HP**, Foz AM, Rabelo Mde S, Gomes GH, Orlandi M, Suvan J, D'Aiuto F, Romito GA. Periodontal therapy and systemic inflammation in type 2 diabetes mellitus: a meta-analysis. *PLoS One* 2015; **10**: e0128344 [PMID: 26010492 DOI: 10.1371/journal.pone.0128344]
- 169 **Janket SJ**, Wightman A, Baird AE, Van Dyke TE, Jones JA. Does periodontal treatment improve glycemic control in diabetic patients? A meta-analysis of intervention studies. *J Dent Res* 2005; **84**: 1154-1159 [PMID: 16304446]
- 170 **Darré L**, Vergnes JN, Gourdy P, Sixou M. Efficacy of periodontal treatment on glycaemic control in diabetic patients: A meta-analysis of interventional studies. *Diabetes Metab* 2008; **34**: 497-506 [PMID: 18948050 DOI: 10.1016/j.diabet.2008.03.006]
- 171 **Garcia R**. Periodontal treatment could improve glycaemic control in diabetic patients. *Evid Based Dent* 2009; **10**: 20-21 [PMID: 19322226 DOI: 10.1038/sj.ebd.6400633]
- 172 **Teeuw WJ**, Gerdes VE, Loos BG. Effect of periodontal treatment on glycaemic control of diabetic patients: a systematic review and meta-analysis. *Diabetes Care* 2010; **33**: 421-427 [PMID: 20103557 DOI: 10.2337/dc09-1378]
- 173 **Vergnes JN**. Treating periodontal disease may improve metabolic control in diabetics. *Evid Based Dent* 2010; **11**: 73-74 [PMID: 20938470 DOI: 10.1038/sj.ebd.6400734]
- 174 **Simpson TC**, Needleman I, Wild SH, Moles DR, Mills EJ. Treatment of periodontal disease for glycaemic control in people with diabetes. *Cochrane Database Syst Rev* 2010; **(5)**: CD004714 [PMID: 20464734 DOI: 10.1002/14651858.CD004714.pub2]
- 175 **Sgolastra F**, Severino M, Pietropaoli D, Gatto R, Monaco A. Effectiveness of periodontal treatment to improve metabolic control in patients with chronic periodontitis and type 2 diabetes: a meta-analysis of randomized clinical trials. *J Periodontol* 2013; **84**: 958-973 [PMID: 23106512 DOI: 10.1902/jop.2012.120377]
- 176 **Liew AK**, Punnathinont N, Lee YC, Yang J. Effect of non-surgical periodontal treatment on HbA1c: a meta-analysis of randomized controlled trials. *Aust Dent J* 2013; **58**: 350-357 [PMID: 23981218 DOI: 10.1111/adj.12091]
- 177 **Corbella S**, Francetti L, Taschieri S, De Siena F, Fabbro MD. Effect of periodontal treatment on glycemic control of patients with diabetes: A systematic review and meta-analysis. *J Diabetes Investig* 2013; **4**: 502-509 [PMID: 24843701 DOI: 10.1111/jdi.12088]
- 178 **Engelbreton S**, Kocher T. Evidence that periodontal treatment improves diabetes outcomes: a systematic review and meta-analysis. *J Periodontol* 2013; **84**: S153-S169 [PMID: 23631575 DOI: 10.1902/jop.2013.1340017]
- 179 **Wang X**, Han X, Guo X, Luo X, Wang D. The effect of periodontal treatment on hemoglobin a1c levels of diabetic patients: a systematic review and meta-analysis. *PLoS One* 2014; **9**: e108412 [PMID: 25255331 DOI: 10.1371/journal.pone.0108412]
- 180 **Sun QY**, Feng M, Zhang MZ, Zhang YQ, Cao MF, Bian LX, Guan QB, Song KL. Effects of periodontal treatment on glycemic control in type 2 diabetic patients: a meta-analysis of randomized controlled trials. *Chin J Physiol* 2014; **57**: 305-314 [PMID: 25575518 DOI: 10.4077/CJP.2014.BAC262]
- 181 **Engelbreton S**. Periodontal disease and glycemic control in diabetics. *Evid Based Dent* 2014; **15**: 93-94 [PMID: 25343401 DOI: 10.1038/sj.ebd.6401040]
- 182 **Li Q**, Hao S, Fang J, Xie J, Kong XH, Yang JX. Effect of non-surgical periodontal treatment on glycemic control of patients with diabetes: a meta-analysis of randomized controlled trials. *Trials* 2015; **16**: 291 [PMID: 26137892 DOI: 10.1186/s13063-015-0810-2]
- 183 **Waugh N**, Cummins E, Royle P, Clar C, Marien M, Richter B, Philip S. Newer agents for blood glucose control in type 2 diabetes: systematic review and economic evaluation. *Health Technol Assess* 2010; **14**: 1-248 [PMID: 20646668 DOI: 10.3310/hta14360]
- 184 **Vasilakou D**, Karagiannis T, Athanasiadou E, Mainou M, Liakos A, Bekiari E, Sarigianni M, Matthews DR, Tsapas A. Sodium-glucose cotransporter 2 inhibitors for type 2 diabetes: a systematic review and meta-analysis. *Ann Intern Med* 2013; **159**: 262-274 [PMID:

- 24026259 DOI: 10.7326/0003-4819-159-4-201308200-00007]
- 185 **Stratton IM**, Adler AI, Neil HA, Matthews DR, Manley SE, Cull CA, Hadden D, Turner RC, Holman RR. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *BMJ* 2000; **321**: 405-412 [PMID: 10938048]
- 186 **Brietzke SA**. Oral antihyperglycemic treatment options for type 2 diabetes mellitus. *Med Clin North Am* 2015; **99**: 87-106 [PMID: 25456645 DOI: 10.1016/j.mcna.2014.08.012]
- 187 **Nauck M**, Frid A, Hermansen K, Shah NS, Tankova T, Mitha IH, Zdravkovic M, Düring M, Matthews DR. Efficacy and safety comparison of liraglutide, glimepiride, and placebo, all in combination with metformin, in type 2 diabetes: the LEAD (liraglutide effect and action in diabetes)-2 study. *Diabetes Care* 2009; **32**: 84-90 [PMID: 18931095 DOI: 10.2337/dc08-1355]
- 188 **Carrera Boada CA**, Martínez-Moreno JM. Current medical treatment of diabetes type 2 and long term morbidity: how to balance efficacy and safety? *Nutr Hosp* 2013; **28** Suppl 2: 3-13 [PMID: 23834040 DOI: 10.3305/nh.2013.28.sup2.6707]
- 189 **Dormandy JA**, Charbonnel B, Eckland DJ, Erdmann E, Massi-Benedetti M, Moules IK, Skene AM, Tan MH, Lefèbvre PJ, Murray GD, Standl E, Wilcox RG, Wilhelmsen L, Betteridge J, Birkeland K, Golay A, Heine RJ, Korányi L, Laakso M, Mokán M, Norkus A, Pirags V, Podar T, Scheen A, Scherbaum W, Scherthaner G, Schmitz O, Skrha J, Smith U, Taton J. Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial In macroVascular Events): a randomised controlled trial. *Lancet* 2005; **366**: 1279-1289 [PMID: 16214598]
- 190 **Colhoun HM**, Livingstone SJ, Looker HC, Morris AD, Wild SH, Lindsay RS, Reed C, Donnan PJ, Guthrie B, Leese GP, McKnight J, Pearson DW, Pearson E, Petrie JR, Philip S, Sattar N, Sullivan FM, McKeigue P. Hospitalised hip fracture risk with rosiglitazone and pioglitazone use compared with other glucose-lowering drugs. *Diabetologia* 2012; **55**: 2929-2937 [PMID: 22945303 DOI: 10.1007/s00125-012-2668-0]
- 191 **Chino Y**, Samukawa Y, Sakai S, Nakai Y, Yamaguchi J, Nakanishi T, Tamai I. SGLT2 inhibitor lowers serum uric acid through alteration of uric acid transport activity in renal tubule by increased glycosuria. *Biopharm Drug Dispos* 2014; **35**: 391-404 [PMID: 25044127 DOI: 10.1002/bdd.1909]
- 192 **Nyirjesy P**, Sobel JD, Fung A, Mayer C, Capuano G, Ways K, Usiskin K. Genital mycotic infections with canagliflozin, a sodium glucose co-transporter 2 inhibitor, in patients with type 2 diabetes mellitus: a pooled analysis of clinical studies. *Curr Med Res Opin* 2014; **30**: 1109-1119 [PMID: 24517339 DOI: 10.1185/03007995.2014.890925]
- 193 **Bruno G**, Landi A. Epidemiology and costs of diabetes. *Transplant Proc* 2011; **43**: 327-329 [PMID: 21335215 DOI: 10.1016/j.transproceed.2010.09.098]
- 194 **Inzucchi SE**, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, Peters AL, Tsapas A, Wender R, Matthews DR. Management of hyperglycemia in type 2 diabetes, 2015: a patient-centered approach: update to a position statement of the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care* 2015; **38**: 140-149 [PMID: 25538310 DOI: 10.2337/dc14-2441]
- 195 **Slots J**. Low-cost periodontal therapy. *Periodontol 2000* 2012; **60**: 110-137 [PMID: 22909110 DOI: 10.1111/j.1600-0757.2011.00429.x]
- 196 **Rad GS**, Bakht LA, Feizi A, Mohebi S. Importance of social support in diabetes care. *J Educ Health Promot* 2013; **2**: 62 [PMID: 24520558 DOI: 10.4103/2277-9531.120864]
- 197 **Dennis CL**. Peer support within a health care context: a concept analysis. *Int J Nurs Stud* 2003; **40**: 321-332 [PMID: 12605954]
- 198 **Qi L**, Liu Q, Qi X, Wu N, Tang W, Xiong H. Effectiveness of peer support for improving glycaemic control in patients with type 2 diabetes: a meta-analysis of randomized controlled trials. *BMC Public Health* 2015; **15**: 471 [PMID: 25943398 DOI: 10.1186/s12889-015-1798-y]
- 199 **Jeffcoat MK**, Jeffcoat RL, Gladowski PA, Bramson JB, Blum JJ. Impact of periodontal therapy on general health: evidence from insurance data for five systemic conditions. *Am J Prev Med* 2014; **47**: 166-174 [PMID: 24953519 DOI: 10.1016/j.amepre.2014.04.001]
- 200 **Petersen PE**, Bourgeois D, Bratthall D, Ogawa H. Oral health information systems--towards measuring progress in oral health promotion and disease prevention. *Bull World Health Organ* 2005; **83**: 686-693 [PMID: 16211160]
- 201 **Axelsson P**, Albandar JM, Rams TE. Prevention and control of periodontal diseases in developing and industrialized nations. *Periodontol 2000* 2002; **29**: 235-246 [PMID: 12102711]
- 202 **Aggarwal A**, Panat SR. Oral health behavior and HbA1c in Indian adults with type 2 diabetes. *J Oral Sci* 2012; **54**: 293-301 [PMID: 23221154]
- 203 **Bowyer V**, Sutcliffe P, Ireland R, Lindenmeyer A, Gadsby R, Graveney M, Sturt J, Dale J. Oral health awareness in adult patients with diabetes: a questionnaire study. *Br Dent J* 2011; **211**: E12 [PMID: 21941301 DOI: 10.1038/sj.bdj.2011.769]
- 204 **Al Habashneh R**, Khader Y, Hammad MM, Almuradi M. Knowledge and awareness about diabetes and periodontal health among Jordanians. *J Diabetes Complications* 2010; **24**: 409-414 [PMID: 19628414 DOI: 10.1016/j.jdiacomp.2009.06.001]
- 205 **Weinspach K**, Staufienbiel I, Memenga-Nicksch S, Ernst S, Geurtsen W, Günay H. Level of information about the relationship between diabetes mellitus and periodontitis--results from a nationwide diabetes information program. *Eur J Med Res* 2013; **18**: 6 [PMID: 23497572 DOI: 10.1186/2047-783X-18-6]
- 206 **Yuen HK**, Wiegand RE, Slate EH, Magruder KM, Salinas CF, London SD. Dental health knowledge in a group of Black adolescents living in rural South Carolina. *J Allied Health* 2008; **37**: 15-21 [PMID: 18444435]
- 207 **Yuen HK**, Wolf BJ, Bandyopadhyay D, Magruder KM, Salinas CF, London SD. Oral health knowledge and behavior among adults with diabetes. *Diabetes Res Clin Pract* 2009; **86**: 239-246 [PMID: 19800143 DOI: 10.1016/j.diabres.2009.09.010]
- 208 **Sandberg GE**, Sundberg HE, Wikblad KF. A controlled study of oral self-care and self-perceived oral health in type 2 diabetic patients. *Acta Odontol Scand* 2001; **59**: 28-33 [PMID: 11318042 DOI: 10.1080/000163501300035742]
- 209 **Tomar SL**, Lester A. Dental and other health care visits among U.S. adults with diabetes. *Diabetes Care* 2000; **23**: 1505-1510 [PMID: 11023144 DOI: 10.2337/diacare.23.10.1505]
- 210 **Bjelland S**, Bray J, Gupta N, Hirscht R. Dentists, diabetes and periodontitis. *Aust Dent J* 2002; **47**: 202-207; quiz 272 [PMID: 12405458 DOI: 10.1111/j.1834-7819.2002.tb00329.x]
- 211 **Saengtipbovorn S**, Taneepanichskul S. Effectiveness of lifestyle change plus dental care (LDCD) program on improving glycemic and periodontal status in the elderly with type 2 diabetes. *BMC Oral Health* 2014; **14**: 72 [PMID: 24934646 DOI: 10.1186/1472-6831-14-72]
- 212 **Dale J**, Lindenmeyer A, Lynch E, Sutcliffe P. Oral health: a neglected area of routine diabetes care? *Br J Gen Pract* 2014; **64**: 103-104 [PMID: 24567612 DOI: 10.3399/bjgp14X677301]
- 213 **Noar SM**. A 10-year retrospective of research in health mass media campaigns: where do we go from here? *J Health Commun* 2006; **11**: 21-42 [PMID: 16546917]
- 214 **Pal BR**. Social media for diabetes health education - inclusive or exclusive? *Curr Diabetes Rev* 2014; **10**: 284-290 [PMID: 25316149]
- 215 **Tokuda Y**, Fujii S, Jimba M, Inoguchi T. The relationship between trust in mass media and the healthcare system and individual health: evidence from the AsiaBarometer Survey. *BMC Med* 2009; **7**: 4 [PMID: 19161600 DOI: 10.1186/1741-7015-7-4]
- 216 **DeSouza SI**, Rashmi MR, Vasanthi AP, Joseph SM, Rodrigues R. Mobile phones: the next step towards healthcare delivery in rural India? *PLoS One* 2014; **9**: e104895 [PMID: 25133610 DOI: 10.1371/journal.pone.0104895]
- 217 **Cole-Lewis H**, Kershaw T. Text messaging as a tool for behavior change in disease prevention and management. *Epidemiol Rev*

- 2010; **32**: 56-69 [PMID: 20354039 DOI: 10.1093/epirev/mxq004]
- 218 **Fjeldsoe BS**, Marshall AL, Miller YD. Behavior change interventions delivered by mobile telephone short-message service. *Am J Prev Med* 2009; **36**: 165-173 [PMID: 19135907 DOI: 10.1016/j.amepre.2008.09.040]
- 219 **Priyaa S**, Murthy S, Sharan S, Mohan K, Joshi A. A pilot study to assess perceptions of using SMS as a medium for health information in a rural setting. *Technol Health Care* 2014; **22**: 1-11 [PMID: 24284551 DOI: 10.3233/THC-130766]
- 220 **Hall CS**, Fottrell E, Wilkinson S, Byass P. Assessing the impact of mHealth interventions in low- and middle-income countries--what has been shown to work? *Glob Health Action* 2014; **7**: 25606 [PMID: 25361730 DOI: 10.3402/gha.v7.25606]
- 221 **Hamine S**, Gerth-Guyette E, Faulx D, Green BB, Ginsburg AS. Impact of mHealth chronic disease management on treatment adherence and patient outcomes: a systematic review. *J Med Internet Res* 2015; **17**: e52 [PMID: 25803266 DOI: 10.2196/jmir.3951]
- 222 **Greene JA**, Choudhry NK, Kilabuk E, Shrank WH. Online social networking by patients with diabetes: a qualitative evaluation of communication with Facebook. *J Gen Intern Med* 2011; **26**: 287-292 [PMID: 20945113 DOI: 10.1007/s11606-010-1526-3]
- 223 **Sayers SL**, Riegel B, Pawlowski S, Coyne JC, Samaha FF. Social support and self-care of patients with heart failure. *Ann Behav Med* 2008; **35**: 70-79 [PMID: 18347906 DOI: 10.1007/s12160-007-9003-x]
- 224 **Demidowich AP**, Lu K, Tamler R, Bloomgarden Z. An evaluation of diabetes self-management applications for Android smartphones. *J Telemed Telecare* 2012; **18**: 235-238 [PMID: 22604278 DOI: 10.1258/jtt.2012.111002]
- 225 **Holtz B**, Lauckner C. Diabetes management via mobile phones: a systematic review. *Telemed J E Health* 2012; **18**: 175-184 [PMID: 22356525 DOI: 10.1089/tmj.2011.0119]
- 226 **Kim YJ**, Rhee SY, Byun JK, Park SY, Hong SM, Chin SO, Chon S, Oh S, Woo JT, Kim SW, Kim YS. A Smartphone Application Significantly Improved Diabetes Self-Care Activities with High User Satisfaction. *Diabetes Metab J* 2015; **39**: 207-217 [PMID: 26124991 DOI: 10.4093/dmj.2015.39.3.207]
- 227 **Chung YS**, Kim Y, Lee CH. Effectiveness of the smart care service for diabetes management. *Healthc Inform Res* 2014; **20**: 288-294 [PMID: 25405065 DOI: 10.4258/hir.2014.20.4.288]
- 228 **Wayne N**, Ritvo P. Smartphone-enabled health coach intervention for people with diabetes from a modest socioeconomic strata community: single-arm longitudinal feasibility study. *J Med Internet Res* 2014; **16**: e149 [PMID: 24907918 DOI: 10.2196/jmir.3180]
- 229 **Choi SH**. A new role for periodontists in the 21st century. *J Periodontal Implant Sci* 2011; **41**: 261-262 [PMID: 22324002 DOI: 10.5051/jpis.2011.41.6.261]
- 230 **Albert DA**, Ward A, Allweiss P, Graves DT, Knowler WC, Kunzel C, Leibel RL, Novak KF, Oates TW, Papapanou PN, Schmidt AM, Taylor GW, Lamster IB, Lalla E. Diabetes and oral disease: implications for health professionals. *Ann N Y Acad Sci* 2012; **1255**: 1-15 [PMID: 22409777 DOI: 10.1111/j.1749-6632.2011.06460.x]

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## Glycosaminoglycan remodeling during diabetes and the role of dietary factors in their modulation

Vemana Gowd, Abhignan Gurukar, Nandini D Chilkunda

Vemana Gowd, Abhignan Gurukar, Nandini D Chilkunda,  
 Department of Molecular Nutrition, CSIR-Central Food  
 Technological Research Institute, Mysore 570 020, India

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**Correspondence to:** Nandini D Chilkunda, PhD, Depart-  
 ment of Molecular Nutrition, CSIR-Central Food Technological  
 Research Institute, Mysore 570 020, India. [cdnandini@cftri.res.in](mailto:cdnandini@cftri.res.in)  
 Telephone: +91-821-2514192

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### Abstract

Glycosaminoglycans (GAGs) play a significant role in  
 various aspects of cell physiology. These are complex  
 polymeric molecules characterized by disaccharides  
 comprising of uronic acid and amino sugar. Compounded  
 to the heterogeneity, these are variously sulfated and  
 epimerized depending on the class of GAG. Among the

various classes of GAG, namely, chondroitin/dermatan  
 sulfate, heparin/heparan sulfate, keratan sulfate and  
 hyaluronic acid (HA), only HA is non-sulfated. GAGs are  
 known to undergo remodeling in various tissues during  
 various pathophysiological conditions, diabetes mellitus  
 being one among them. These changes will likely affect  
 their structure thereby impinging on their functionality.  
 Till date, diabetes has been shown to affect GAGs in  
 organs such as kidney, liver, aorta, skin, erythrocytes,  
*etc.* to name a few, with deleterious consequences. One  
 of the mainstays in the treatment of diabetes is through  
 dietary means. Various dietary factors are known to  
 play a significant role in regulating glucose homeostasis.  
 Furthermore, in recent years, there has been a keen  
 interest to decipher the role of dietary factors on GAG  
 metabolism. This review focuses on the remodeling  
 of GAGs in various organs during diabetes and their  
 modulation by dietary factors. While effect of diabetes  
 on GAG metabolism has been worked out quite a bit,  
 studies on the role of dietary factors in their modulation  
 has been few and far between. We have tried our best  
 to give the latest reports available on this subject.

**Key words:** Glycosaminoglycans; Diabetes; Proteoglycans;  
 Remodeling; Dietary factors

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**Core tip:** Glycosaminoglycans/Proteoglycans are  
 important polymeric molecules which play important  
 roles in cell physiology. Under pathological conditions  
 such as diabetes, they are known to undergo remodeling  
 affecting their structure-function relationship. This  
 review article deals with its remodeling in various tissues  
 and their modulation by dietary factors.

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 remodeling during diabetes and the role of dietary factors in their  
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## INTRODUCTION

Diabetes mellitus, henceforth referred as diabetes, is a disorder characterized by sustained hyperglycemia. According to World Health Organization studies, diabetes could be one of the leading causes of death in world population by 2030<sup>[1]</sup>. Approximately 80% of the deaths due to diabetes are reported to have occurred in low- and middle-income countries<sup>[2]</sup>. Diabetes could occur as a result of either decreased insulin secretion by pancreatic beta cells (Type 1 diabetes) or as a result of insulin resistance (Type 2 diabetes)<sup>[3]</sup>. In both these instances, glucose uptake by the cells and their disposal is affected. Insulin is required for storing glucose as glycogen in the liver and skeletal muscles. Lack of insulin secretion/insulin sensitivity, alter the glycogenolysis and gluconeogenesis results in the subsequent increase in blood glucose<sup>[4]</sup>.

Sustained hyperglycemia in the long run results in the manifestation of micro- and macro-vascular complications. Nephropathy, retinopathy, and neuropathy are some of the serious secondary complications of diabetes which are known to affect kidney, eye and nerves, respectively<sup>[5]</sup>. In both micro- and macro-vascular complications, extracellular matrix components (ECM) are affected. ECM is composed of proteoglycans (PGs) and glycoproteins. PGs are complex polymeric molecules consisting of core proteins to which are attached the glycosaminoglycan (GAG) chains. They play an important role in various aspects of cell growth and behavior not only by acting as a supporting structure but also as a depot for growth factors and other signaling molecules. Changes in the structure-function relationship of GAGs/PGs impinge on their biological activities and influence the functioning of organ and the organ systems<sup>[6]</sup>.

Controlling blood sugar levels will have a positive impact on the health of the organism. It can be managed by drugs, diet and exercise with positive outcomes. Diet, in particular, has been demonstrated to play a vital role in the management of diabetes. In recent years, with increasing emphasis being placed on functional foods, a lot of research efforts are being directed towards elucidating and deciphering novel bioactive molecules from dietary sources that could be used in tailoring functional foods. These foods are expected to increase the general wellness as well as attenuate the disease condition by modulating various processes.

## GAGS- STRUCTURE AND BIOSYNTHESIS

GAGs are polymeric molecules that are present in all cells. There are 4 classes of GAGs known till date

namely; hyaluronic acid (HA), chondroitin sulfate/dermatan sulfate (CS/DS), heparan sulfate/heparin (HS/Hep) and keratan sulfate (KS). CS/DS and HS/Hep are synthesized on a core protein through the linkage region tetrasaccharide of Xylose-Galactose-Galactose-Glucuronic acid. They contain repeating disaccharide units of uronic acid and amino sugar which are variously sulfated<sup>[7]</sup>. Disaccharides of CS/DS are glucuronic acid/iduronic acid and N-acetyl-D galactosamine and disaccharides of HS/Hep are glucuronic acid/Iduronic acid and N-acetyl-D glucosamine. The composite structure comprising of GAGs with core proteins are known as PGs. There are a few exceptions: HA which is composed of glucuronic acid and N-acetyl-D-glucosamine is not attached to the core proteins, and KS does not contain an uronic acid but contains galactose instead. KS chains are attached to core proteins *via* asparagine (N-linked) or serine/threonine (O-linked)<sup>[8]</sup>. All the classes of GAGs except for HA are variously sulfated.

Biosynthesis of all classes of GAGs with the exception of HA is synthesized in endoplasmic reticulum and Golgi. HA, is however synthesized by transmembrane hyaluronan synthases (HAS1, HAS2, and HAS3)<sup>[9]</sup>. Biosynthesis of GAGs is an energy-intensive process. Interestingly, one of the disaccharides, N-Acetyl-D-glucosamine is synthesized through hexosamine biosynthetic pathway that is also known as nutrient sensor pathway<sup>[10]</sup>.

GAGs play vital roles in various aspects of cell physiology which brings about cell growth and development<sup>[11]</sup>. They can bring about biological activities by forming binding domains on growth factors and other proteins. GAGs undergo remodeling under various pathological conditions bringing about changes in their structure and function. In conditions of diabetes, changes in GAG structure and function have been observed in different tissues.

## DIABETIC NEPHROPATHY AND GAGS

Nephropathy is one of the major secondary complications of diabetes which, in the long run, leads to end-stage renal failure. It is characterized by increased deposition of ECM components. ECM components maintain the integrity of the cell and their interactions with GAGs are essential for maintaining the extracellular morphology and cell adhesion<sup>[12]</sup>. The increase in the ECM components in kidney leads to the thickening of basement membrane and expansion of glomerular mesangial matrix, thereby affecting the filtration process. PGs/GAGs are the part of ECM components, and it is experimentally proved that PGs/GAGs are altered during diabetic nephropathy<sup>[13]</sup>. DN is marked by albuminuria and overt proteinuria at later stages. Earlier studies have implicated loss of heparan sulfate on the basement membrane as one of the reasons for leakage of proteins<sup>[13]</sup>. It was further supported by biopsies from patients with diabetes, in which structural modifications in HS GAGs was observed<sup>[14]</sup>. GAGs in the basement

membrane, especially HS are known to influence the permselectivity. This however, has been discounted by injecting heparinase, an enzyme which cleaves Hep/HS, intravenously which prevented albuminuria despite a decrease in HS chains<sup>[15]</sup>. Recent evidence further show that CS along with HA play equally important role in charge selectivity of the glomerular membrane<sup>[16]</sup>. Kidney GAGs comprises about 86% HS and 14% CS/DS among sulfated GAGs in adults whereas CS comprises 75% in embryonic kidney<sup>[17]</sup>. Studies on Streptozotocin-induced diabetic rats have shown that there is significant qualitative and quantitative changes in kidney GAGs. CS/DS from kidney was shown to be altered structurally which impinged on their functionality tested in terms of binding to laminin, fibronectin and type IV collagen<sup>[18]</sup>. Further, it has been observed that heparanase, an endosulfatase is involved in the pathogenesis of DN<sup>[19]</sup>.

Multiple factors have been implicated in causation of diabetic nephropathy. Advanced glycation end products (AGEs) and oxidative stress has been considered as one of the major factors<sup>[20,21]</sup>. AGEs are the heterogeneous group of molecules which are formed *via* Maillard reactions from the non-enzymatic reaction of reducing sugars with free amino groups of proteins, lipids and nucleic acids<sup>[22]</sup>. Accumulation of AGEs in patients with diabetic nephropathy is due to enhanced formation and decreased the clearance of AGEs<sup>[23]</sup>. AGEs tend to alter properties of large matrix proteins like laminin, collagen, fibronectin and vitronectin, through AGE-AGE intermolecular covalent bonds or crosslinking with these proteins<sup>[23]</sup>. Formation of AGEs on laminin reduced polymer elongation as well as reduced binding of laminin to type IV collagen and HSPGs<sup>[24,25]</sup>. AGEs also play a significant role in thickening of GBM and mesangial expansions that are considered to be hallmarks of diabetic nephropathy because AGEs formation on ECM proteins dysregulates their degradation by matrix metalloproteinase<sup>[26,27]</sup>. AGEs are also implicated in the increased production of TGF $\beta$ , a cytokine which is responsible for increased synthesis of ECM components in the kidney<sup>[28-30]</sup>. However, the mechanism by which GAGs/PGs are modulated in the diabetic kidney has not been deciphered.

## LIVER GAGS IN DIABETES

The liver is an organ of great metabolic importance. It is rich in GAGs and harbors CS/DS, HS, HA and KS<sup>[31,32]</sup>. Changes in liver GAGs have been observed during various physiological and pathological conditions such as diabetes<sup>[33]</sup>, hypercholesterolemia<sup>[34]</sup>, liver cirrhosis<sup>[35]</sup>, and cholestasis<sup>[36]</sup>, *etc.*, to name a few. Diabetes is known to deregulate lipid metabolism and GAGs, in particular the HS class. It plays an important role in lipoprotein metabolism in liver by acting as a receptor or a co-receptor along with LDL-receptor, LRP and ApoE<sup>[37,38]</sup>. HSPGs such as syndecan-1 and perlecan, in particular, have been implicated in lipoprotein metabolism, and it

is evidenced by impairment in the clearance of remnant lipoproteins in syndecan-1 knockout mice<sup>[39]</sup>. HS-GAGs are also known to be involved in hepatic clearance of apoB-48-containing lipoproteins<sup>[40]</sup>. During experimental diabetic conditions reduced N-sulfation has been observed in liver HS GAG as compared to control<sup>[33]</sup>. This was determined to be due to decreased glucosaminyl N-deacetylase activity and N-sulfotransferase activities in hepatocytes<sup>[41]</sup>. Decreased content of liver HS GAGs as a result of decreased HSPGs was associated with the decreased postprandial clearance of apoB-48-containing lipoproteins. It has been determined that decrease in HSPG perlecan was associated with the delayed clearance of apoB-48-containing lipoproteins<sup>[40]</sup>. However, Bishop *et al.*<sup>[42]</sup> have shown that decrease in lipoprotein clearance during diabetes is not due to changes in HS as no differences were observed between normal and diabetic littermates in liver heparan sulfate content, sulfation and syndecan-1 protein levels. Some of the degradative enzymes of HS have been observed to affect the metabolism of lipoproteins. Noted amongst them is Sulf 2 which encodes heparan sulfate glucosamine- 6-O-endosulfatase 2 which is responsible for the degradation of HSPGs by removing 6-O sulfate groups. Involvement of HSPGs in hepatic clearance was further evidenced by the deletion of SULF2 in cultures hepatocytes. Knockdown of SULF2 showcased the increased HSPG-mediated catabolism of remnant lipoproteins in cultured cells<sup>[43]</sup>.

Matrix PGs in the liver are affected by insulin and fatty acids. In a study conducted by Olsson *et al.*<sup>[44]</sup>, it was observed that insulin and non-esterified fatty acids modulate PG synthesis in hepatic cells so much so that the changes in PG composition affected their binding to remnant B-VLDL particles contributing to dyslipidemia of insulin resistance.

## EFFECT OF DIABETES ON AORTIC GAGS

Cardio-vascular disease is one of the major complications of diabetes. Arterial walls are rich in PGs and are implicated in the pathogenesis of atherosclerosis by virtue of their ability to bind and trap LDL. In a study carried out in diabetic monkeys, it was observed that diabetes resulted in increased DS class of GAGs in arteries which was positively correlated with tissue cholesterol promoting atherosclerosis<sup>[45]</sup>. Three major CS/DSPGs present in the arterial wall are versican, decorin and biglycan<sup>[46]</sup>. In aortic endothelial cells, high glucose condition resulted in decreased perlecan level indicating remodeling of PGs<sup>[47]</sup>. Studies on BAEC suggested that reduced sulfate incorporation in the HSPGs<sup>[48]</sup>.

Factors affecting the synthesis and degradation of PGs in the aorta as a result of diabetes have not been critically studied. TGF $\beta$  has been one of the factors implicated in changes in PG synthesis. It is known to be produced in hyperglycemic conditions and induces changes in PGs secreted by vascular smooth muscle

cells increasing their propensity to retain and bind lipoproteins in the vascular wall<sup>[49]</sup>.

## EFFECT OF DIABETES ON ERYTHROCYTE GAGS

Diabetes is known to affect erythrocytes by increasing their aggregation binding to endothelial cells and decreasing the deformability<sup>[50]</sup>. Diabetes is also associated with the increase in membrane lipids and changes in its fluidity. Reports on erythrocyte GAGs are scarce. HS expression was observed in human erythrocytes infected with the malarial parasite which helped in the rosette formation with uninfected erythrocytes<sup>[51]</sup>. Furthermore, HS was found to be a mediator for the binding of *Plasmodium falciparum*-infected erythrocytes to endothelial cells *via* the DBL1 $\alpha$  domain of PfEMP<sup>[52]</sup>. Recent findings from our laboratory revealed the presence of CS/DS class of GAGs in erythrocytes of experimentally-induced diabetic rats. Erythrocytes from diabetic rats had increased levels of CS/DS when compared to age-matched non-diabetic control rats. They appeared to mediate the binding of erythrocytes to ECM<sup>[53]</sup>. Erythrocytes isolated from rats that were diabetic as well as hypercholesterolaemic showed higher binding to ECM components than that isolated from diabetic rats<sup>[54]</sup>. Further work on synthesis and regulation of GAGs in erythrocytes should be able to throw light on the function of these important molecules.

## GAGS IN DIABETIC RETINOPATHY

Retinopathy is one of the secondary complications of diabetes. Diabetic retinopathy leads to vision loss with their associated abnormalities in vascular permeability<sup>[55]</sup>. Changes in the vascular permeability are associated with combination of abnormalities namely: Thickening of basement membrane, leakage of various compounds, capillary occlusion, and formation of new vessels along with fibrous tissue<sup>[56-58]</sup>. Fluorescent microscopic studies have found the presence of HS, CS/DS, HA throughout the retina but the presence of KS is found to be limited to the sclera<sup>[59]</sup>. Studies on the incorporation of [<sup>35</sup>S]- and [<sup>14</sup>C] glucosamine into GAGs that were isolated from retinal vessel basement membrane suggested that HS GAG is the major GAG present in basement membrane<sup>[60]</sup>. Various studies have been conducted to decipher the role of HS GAG in retinopathy. A study on the metabolism of GAGs in retina in streptozotocin-induced diabetic rats found decreased synthesis of HSPGs and was found to be associated with the decreased expression of perlecan<sup>[61]</sup>. It has also been demonstrated that quantitatively more GAGs is found in tears of patients with diabetic retinopathy than in non-diabetic people<sup>[62]</sup>. Furthermore, diabetic retinopathy was associated with the reduced production of HS GAG in the vitreous and increased expression of surface binding exogenous VEGF<sup>[63]</sup>. It is well known that HS

GAGs are recognized as a co-receptor for fibroblast growth factor. FGF is a potent endothelial cell mitogen that has proposed to be involved in the development of proliferative diabetic retinopathy. Changes in the distribution of FGF during diabetes are associated with the development of retinopathy and retinal neovascularization. These studies reveal the possible role of FGF in the development of neovascularization and contribution of HSPGs in it<sup>[64]</sup>. In a study conducted on diabetic subjects, it was observed that there was a correlation between diabetic retinopathy, erythrocyte anionic charge and urinary GAG excretion<sup>[65]</sup>.

## DIABETES AND SKIN GAGS

Despite the fact that skin is rich in GAGs, not much work has been carried out to determine the effect of diabetes on its remodeling. The skin of STZ-induced diabetic rats showed decreased GAG content as a result of decreased GAG biosynthesis<sup>[66]</sup>. The decrease of GAG content in the skin of diabetic rats was earlier reported to be as a result of decreased circulating IGF- I level, increased plasma content of LMW-BPs and increased proteolytic activity of the skin<sup>[66]</sup>.

## ROLE OF DIETARY FACTORS IN MODULATION OF GAGs

Diet plays major roles in the management of diabetes. The information with respect to the role of diet on GAG metabolism is scanty. In a study conducted by Taylor *et al.*<sup>[67]</sup>, it was observed that extracts from some of the plants present in Amazon rain forest stimulated GAG assembly in both wild type and mutant cell line defect in one of the key biosynthetic enzymes-xylosyltransferase. These findings suggest the importance of plant products in modulation of GAGs in animal cells. Various dietary factors have been implicated in attenuating diabetic nephropathy *per se*, but there are very few reports on their effect on GAG metabolism. Fiber-rich sources such as wheat bran and Guar gum altered decreased GAG synthesis in STZ-induced diabetic rats<sup>[68]</sup>. In another study, feeding of bitter melon (*Momordica charantia* LINN) resulted in amelioration of decreased synthesis of GAGs in the kidney of diabetic rats<sup>[69]</sup>. In a similar vein, dietary feeding of *Tinospora cordifolia* resulted in attenuation of decreased CS/DS in STZ-induced diabetic rat kidney<sup>[70]</sup>. Not only in diabetes, even in normal conditions are some of the dietary factors known to affect GAGs. Noted among them, Genistein has been determined to decrease synthesis of GAGs<sup>[71]</sup>. Dietary manganese has been found to affect aortic GAGs in rats by altering composition and sulfation pattern of heparan sulfate GAG<sup>[72]</sup>. Similarly, wild blue berry consumption altered the GAG composition in the aorta<sup>[73]</sup>. In a study conducted, *Annona squamosa* showed beneficial effect on wound healing by increasing the synthesis of GAGs and collagen in STZ-induced diabetic rats<sup>[74]</sup>.

GAG consumption can also occur by consuming foods of animal origin<sup>[75]</sup>. However, not much work has been carried out with respect to its metabolic effects in the body. The mixture of GAGs, called sulodexide has been demonstrated to contain proteinuria and ameliorate markers of diabetic nephropathy in clinical studies<sup>[76]</sup>. It has also been observed that oral administration of high molecular weight hyaluronan controls immune system via Toll-like receptor 4 in the intestinal epithelium<sup>[77]</sup>.

## FUTURE PERSPECTIVES

Despite the fact that rapid strides have been made with respect to deciphering the importance of GAGs in health and disease of the organism, more needs to be done especially with relation to their regulation under various conditions both normal and pathological. Also, evaluating various dietary molecules which could influence GAG metabolism will go a long way in therapeutic applications and development of functional foods.

## REFERENCES

- Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med* 2006; **3**: e442 [PMID: 17132052 DOI: 10.1371/journal.pmed.0030442]
- Klafke A, Duncan BB, Stevens A, Rosa Rdos S, de Moura L, Malta D, Schmidt MI. The decline in mortality due to acute complications of diabetes mellitus in Brazil, 1991-2010. *BMC Public Health* 2015; **15**: 772 [PMID: 26259708 DOI: 10.1186/s12889-015-2123-5]
- Farag YM, Gaballa MR. Diabetes: an overview of a rising epidemic. *Nephrol Dial Transplant* 2011; **26**: 28-35 [PMID: 21045078 DOI: 10.1093/ndt/gfq576]
- Edgerton DS, Cardin S, Emshwiller M, Neal D, Chandramouli V, Schumann WC, Landau BR, Rossetti L, Cherrington AD. Small increases in insulin inhibit hepatic glucose production solely caused by an effect on glycogen metabolism. *Diabetes* 2001; **50**: 1872-1882 [PMID: 11473051 DOI: 10.2337/diabetes.50.8.1872]
- King P, Peacock I, Donnelly R. The UK prospective diabetes study (UKPDS): clinical and therapeutic implications for type 2 diabetes. *Br J Clin Pharmacol* 1999; **48**: 643-648 [PMID: 10594464 DOI: 10.1046/j.1365-2125.1999.00092.x]
- Bülow HE, Hobert O. The molecular diversity of glycosaminoglycans shapes animal development. *Annu Rev Cell Dev Biol* 2006; **22**: 375-407 [PMID: 16805665 DOI: 10.1146/annurev.cellbio.22.010605.093433]
- Sugahara K, Kitagawa H. Recent advances in the study of the biosynthesis and functions of sulfated glycosaminoglycans. *Curr Opin Struct Biol* 2000; **10**: 518-527 [PMID: 11042448 DOI: 10.1016/S0959-440X(00)00125-1]
- Funderburgh JL. Keratan sulfate: structure, biosynthesis, and function. *Glycobiology* 2000; **10**: 951-958 [PMID: 11030741 DOI: 10.1093/glycob/10.10.951]
- Lee JY, Spicer AP. Hyaluronan: a multifunctional, megaDalton, stealth molecule. *Curr Opin Cell Biol* 2000; **12**: 581-586 [PMID: 10978893 DOI: 10.1016/S0955-0674(00)00135-6]
- Zachara NE, Hart GW. O-GlcNAc a sensor of cellular state: the role of nucleocytoplasmic glycosylation in modulating cellular function in response to nutrition and stress. *Biochim Biophys Acta* 2004; **1673**: 13-28 [PMID: 15238246 DOI: 10.1016/j.bbagen.2004.03.016]
- Bishop JR, Schuksz M, Esko JD. Heparan sulphate proteoglycans fine-tune mammalian physiology. *Nature* 2007; **446**: 1030-1037 [PMID: 17460664 DOI: 10.1038/nature05817]
- Barkalow FJ, Schwarzbauer JE. Interactions between fibronectin and chondroitin sulfate are modulated by molecular context. *J Biol Chem* 1994; **269**: 3957-3962 [PMID: 8307950]
- Lewis EJ, Xu X. Abnormal glomerular permeability characteristics in diabetic nephropathy: implications for the therapeutic use of low-molecular weight heparin. *Diabetes Care* 2008; **31** Suppl 2: S202-S207 [PMID: 18227486 DOI: 10.2337/dc08-s251]
- Yard BA, Kahlert S, Engelleiter R, Resch S, Waldherr R, Groffen AJ, van den Heuvel LP, van der Born J, Berden JH, Kröger S, Hafner M, van der Woude FJ. Decreased glomerular expression of agrin in diabetic nephropathy and podocytes, cultured in high glucose medium. *Exp Nephrol* 2001; **9**: 214-222 [PMID: 11340306 DOI: 10.1159/000052614]
- Wijnhoven TJ, Lensen JF, Wismans RG, Lefeber DJ, Rops AL, van der Vlag J, Berden JH, van den Heuvel LP, van Kuppevelt TH. Removal of heparan sulfate from the glomerular basement membrane blocks protein passage. *J Am Soc Nephrol* 2007; **18**: 3119-3127 [PMID: 18003778 DOI: 10.1681/ASN.2007020198]
- Jeansson M, Haraldsson B. Glomerular size and charge selectivity in the mouse after exposure to glucosaminoglycan-degrading enzymes. *J Am Soc Nephrol* 2003; **14**: 1756-1765 [PMID: 12819235 DOI: 10.1097/01.ASN.0000072742.02714.6E]
- Steer DL, Shah MM, Bush KT, Stuart RO, Sampogna RV, Meyer TN, Schwesinger C, Bai X, Esko JD, Nigam SK. Regulation of ureteric bud branching morphogenesis by sulfated proteoglycans in the developing kidney. *Dev Biol* 2004; **272**: 310-327 [PMID: 15282150 DOI: 10.1016/j.ydbio.2004.04.029]
- Joladarashi D, Salimath PV, Chilkunda ND. Diabetes results in structural alteration of chondroitin sulfate/dermatan sulfate in the rat kidney: effects on the binding to extracellular matrix components. *Glycobiology* 2011; **21**: 960-972 [PMID: 21406563 DOI: 10.1093/glycob/cwr029]
- Maxhimer JB, Somenek M, Rao G, Pesce CE, Baldwin D, Gattuso P, Schwartz MM, Lewis EJ, Prinz RA, Xu X. Heparanase-1 gene expression and regulation by high glucose in renal epithelial cells: a potential role in the pathogenesis of proteinuria in diabetic patients. *Diabetes* 2005; **54**: 2172-2178 [PMID: 15983219 DOI: 10.2337/diabetes.54.7.2172]
- Yamagishi S, Matsui T. Advanced glycation end products, oxidative stress and diabetic nephropathy. *Oxid Med Cell Longev* 2010; **3**: 101-108 [PMID: 20716934 DOI: 10.4161/oxim.3.2.11148]
- Thomas MC, Forbes JM, Cooper ME. Advanced glycation end products and diabetic nephropathy. *Am J Ther* 2005; **12**: 562-572 [PMID: 16280650]
- Vlassara H, Palace MR. Diabetes and advanced glycation endproducts. *J Intern Med* 2002; **251**: 87-101 [PMID: 11905595 DOI: 10.1046/j.1365-2796.2002.00932.x]
- Oleniuc M, Secara I, Onofriescu M, Hogas S, Voroneanu L, Siroopol D, Covic A. Consequences of Advanced Glycation End Products Accumulation in Chronic Kidney Disease and Clinical Usefulness of Their Assessment Using a Non-invasive Technique - Skin Autofluorescence. *Maedica (Buchar)* 2011; **6**: 298-307 [PMID: 22879845]
- Charonis AS, Reger LA, Dege JE, Kouzi-Koliakos K, Furcht LT, Wohlhueter RM, Tsilibary EC. Laminin alterations after in vitro nonenzymatic glycosylation. *Diabetes* 1990; **39**: 807-814 [PMID: 2113013]
- Charonis AS, Tsilibary EC. Structural and functional changes of laminin and type IV collagen after nonenzymatic glycation. *Diabetes* 1992; **41** Suppl 2: 49-51 [PMID: 1526336]
- Krishnamurti U, Rondeau E, Sraer JD, Michael AF, Tsilibary EC. Alterations in human glomerular epithelial cells interacting with nonenzymatically glycosylated matrix. *J Biol Chem* 1997; **272**: 27966-27970 [PMID: 9346947 DOI: 10.1074/jbc.272.44.27966]
- Ishibashi Y, Yamagishi S, Matsui T, Ohta K, Tanoue R, Takeuchi M, Ueda S, Nakamura K, Okuda S. Pravastatin inhibits advanced glycation end products (AGEs)-induced proximal tubular cell apoptosis and injury by reducing receptor for AGEs (RAGE) level. *Metabolism* 2012; **61**: 1067-1072 [PMID: 22386936 DOI: 10.1016/j.metabol.2012.01.006]
- Border WA, Noble NA. Transforming growth factor beta in tissue fibrosis. *N Engl J Med* 1994; **331**: 1286-1292 [PMID: 7935686 DOI: 10.1056/NEJM199411103311907]



- 29 **Ling H**, Vamvakas S, Busch G, Dämmrich J, Schramm L, Lang F, Heidland A. Suppressing role of transforming growth factor-beta 1 on cathepsin activity in cultured kidney tubule cells. *Am J Physiol* 1995; **269**: F911-F917 [PMID: 8594887]
- 30 **Xiang G**, Schinzel R, Simm A, Münch G, Sebekova K, Kasper M, Niwa T, Schmitz C, Heidland A. Advanced glycation end products (AGEs)-induced expression of TGF-beta 1 is suppressed by a protease in the tubule cell line LLC-PK1. *Nephrol Dial Transplant* 2001; **16**: 1562-1569 [PMID: 11477156 DOI: 10.1093/ndt/16.8.1562]
- 31 **Minami R**, Ikeno T, Igarashi C, Tsugawa S, Nakao T. Characterization of keratan sulfate isolated from liver affected by Morquio syndrome. *Tohoku J Exp Med* 1983; **139**: 321-326 [PMID: 6222514 DOI: 10.1620/tjem.139.321]
- 32 **Gressner AM**, Vasel A. Developmental changes of proteoglycan synthesis in rat liver and isolated hepatocytes. *Mech Ageing Dev* 1985; **31**: 307-327 [PMID: 3934470 DOI: 10.1016/0047-6374(85)90097-1]
- 33 **Kjellén L**, Bielefeld D, Hook M. Reduced sulfation of liver heparan sulfate in experimentally diabetic rats. *Diabetes* 1983; **32**: 337-342 [PMID: 6219905]
- 34 **MacArthur JM**, Bishop JR, Stanford KI, Wang L, Bensadoun A, Witztum JL, Esko JD. Liver heparan sulfate proteoglycans mediate clearance of triglyceride-rich lipoproteins independently of LDL receptor family members. *J Clin Invest* 2007; **117**: 153-164 [PMID: 17200715 DOI: 10.1172/JCI29154]
- 35 **Tátrai P**, Egedi K, Somorácz A, van Kuppevelt TH, Ten Dam G, Lyon M, Deakin JA, Kiss A, Schaff Z, Kovalszky I. Quantitative and qualitative alterations of heparan sulfate in fibrogenic liver diseases and hepatocellular cancer. *J Histochem Cytochem* 2010; **58**: 429-441 [PMID: 20124094 DOI: 10.1369/jhc.2010.955161]
- 36 **Guedes PL**, Castañón MC, Nagaoka MR, Aguiar JA. Increase of glycosaminoglycans and metalloproteinases 2 and 9 in liver extracellular matrix on early stages of extrahepatic cholestasis. *Arq Gastroenterol* 2014; **51**: 309-315 [PMID: 25591159 DOI: 10.1590/S0004-28032014000400008]
- 37 **de Beer F**, Hendriks WL, van Vark LC, Kamerling SW, van Dijk KW, Hofker MH, Smelt AH, Havekes LM. Binding of beta-VLDL to heparan sulfate proteoglycans requires lipoprotein lipase, whereas ApoE only modulates binding affinity. *Arterioscler Thromb Vasc Biol* 1999; **19**: 633-637 [PMID: 10073967 DOI: 10.1161/01.ATV.19.3.633]
- 38 **Williams KJ**, Fless GM, Petrie KA, Snyder ML, Brocia RW, Swenson TL. Mechanisms by which lipoprotein lipase alters cellular metabolism of lipoprotein(a), low density lipoprotein, and nascent lipoproteins. Roles for low density lipoprotein receptors and heparan sulfate proteoglycans. *J Biol Chem* 1992; **267**: 13284-13292 [PMID: 1320015]
- 39 **Stanford KI**, Bishop JR, Foley EM, Gonzales JC, Niesman IR, Witztum JL, Esko JD. Syndecan-1 is the primary heparan sulfate proteoglycan mediating hepatic clearance of triglyceride-rich lipoproteins in mice. *J Clin Invest* 2009; **119**: 3236-3245 [PMID: 19805913 DOI: 10.1172/JCI38251]
- 40 **Ebara T**, Conde K, Kako Y, Liu Y, Xu Y, Ramakrishnan R, Goldberg IJ, Shachter NS. Delayed catabolism of apoB-48 lipoproteins due to decreased heparan sulfate proteoglycan production in diabetic mice. *J Clin Invest* 2000; **105**: 1807-1818 [PMID: 10862796]
- 41 **Unger E**, Pettersson I, Eriksson UJ, Lindahl U, Kjellén L. Decreased activity of the heparan sulfate-modifying enzyme glucosaminyl N-deacetylase in hepatocytes from streptozotocin-diabetic rats. *J Biol Chem* 1991; **266**: 8671-8674 [PMID: 2026583]
- 42 **Bishop JR**, Foley E, Lawrence R, Esko JD. Insulin-dependent diabetes mellitus in mice does not alter liver heparan sulfate. *J Biol Chem* 2010; **285**: 14658-14662 [PMID: 20236939 DOI: 10.1074/jbc.M110.112391]
- 43 **Chen K**, Liu ML, Schaffer L, Li M, Boden G, Wu X, Williams KJ. Type 2 diabetes in mice induces hepatic overexpression of sulfatase 2, a novel factor that suppresses uptake of remnant lipoproteins. *Hepatology* 2010; **52**: 1957-1967 [PMID: 21049473 DOI: 10.1002/hep.23916]
- 44 **Olsson U**, Egnell AC, Lee MR, Lundén GO, Lorentzon M, Salmivirta M, Bondjers G, Camejo G. Changes in matrix proteoglycans induced by insulin and fatty acids in hepatic cells may contribute to dyslipidemia of insulin resistance. *Diabetes* 2001; **50**: 2126-2132 [PMID: 11522680 DOI: 10.2337/diabetes.50.9.2126]
- 45 **Edwards IJ**, Wagner JD, Vogl-Willis CA, Litwak KN, Cefalu WT. Arterial heparan sulfate is negatively associated with hyperglycemia and atherosclerosis in diabetic monkeys. *Cardiovasc Diabetol* 2004; **3**: 6 [PMID: 15117408]
- 46 **Williams KJ**. Arterial wall chondroitin sulfate proteoglycans: diverse molecules with distinct roles in lipoprotein retention and atherogenesis. *Curr Opin Lipidol* 2001; **12**: 477-487 [PMID: 11561166]
- 47 **Vogl-Willis CA**, Edwards IJ. High-glucose-induced structural changes in the heparan sulfate proteoglycan, perlecan, of cultured human aortic endothelial cells. *Biochim Biophys Acta* 2004; **1672**: 36-45 [PMID: 15056491 DOI: 10.1016/j.bbagen.2004.02.005]
- 48 **Humphries DE**, Silbert CK, Silbert JE. Glycosaminoglycan production by bovine aortic endothelial cells cultured in sulfate-depleted medium. *J Biol Chem* 1986; **261**: 9122-9127 [PMID: 3087988]
- 49 **Yang SN**, Burch ML, Tannock LR, Evanko S, Osman N, Little PJ. Transforming growth factor-β regulation of proteoglycan synthesis in vascular smooth muscle: contribution to lipid binding and accelerated atherosclerosis in diabetes. *J Diabetes* 2010; **2**: 233-242 [PMID: 20923499 DOI: 10.1111/j.1753-0407.2010.00089.x]
- 50 **Yedgar S**, Koshkaryev A, Barshtein G. The red blood cell in vascular occlusion. *Pathophysiol Haemost Thromb* 2002; **32**: 263-268 [PMID: 13679654 DOI: 10.1159/000073578]
- 51 **Vogt AM**, Winter G, Wahlgren M, Spillmann D. Heparan sulphate identified on human erythrocytes: a Plasmodium falciparum receptor. *Biochem J* 2004; **381**: 593-597 [PMID: 15209561 DOI: 10.1042/BJ20040762]
- 52 **Vogt AM**, Barragan A, Chen Q, Kironde F, Spillmann D, Wahlgren M. Heparan sulfate on endothelial cells mediates the binding of Plasmodium falciparum-infected erythrocytes via the DBLα domain of PfEMP1. *Blood* 2003; **101**: 2405-2411 [PMID: 12433689 DOI: 10.1182/blood-2002-07-2016]
- 53 **Srikanth CB**, Salimath PV, Nandini CD. Erythrocytes express chondroitin sulphate/dermatan sulphate, which undergoes quantitative changes during diabetes and mediate erythrocyte adhesion to extracellular matrix components. *Biochimie* 2012; **94**: 1347-1355 [PMID: 22426386 DOI: 10.1016/j.biochi.2012.03.002]
- 54 **Gowd V**, Nandini CD. Erythrocytes in the combined milieu of high glucose and high cholesterol shows glycosaminoglycan-dependent cytoadherence to extracellular matrix components. *Int J Biol Macromol* 2015; **73**: 182-188 [PMID: 25475844 DOI: 10.1016/j.ijbiomac.2014.11.019]
- 55 **Scheppke L**, Aguilar E, Gariano RF, Jacobson R, Hood J, Doukas J, Cao J, Noronha G, Yee S, Weis S, Martin MB, Soll R, Cheresch DA, Friedlander M. Retinal vascular permeability suppression by topical application of a novel VEGFR2/Src kinase inhibitor in mice and rabbits. *J Clin Invest* 2008; **118**: 2337-2346 [PMID: 18483622 DOI: 10.1172/JCI33361]
- 56 **Ljubimov AV**, Burgeson RE, Butkowski RJ, Couchman JR, Zardi L, Ninomiya Y, Sado Y, Huang ZS, Nesburn AB, Kenney MC. Basement membrane abnormalities in human eyes with diabetic retinopathy. *J Histochem Cytochem* 1996; **44**: 1469-1479 [PMID: 8985139 DOI: 10.1177/44.12.8985139]
- 57 **Conde-Knape K**. Heparan sulfate proteoglycans in experimental models of diabetes: a role for perlecan in diabetes complications. *Diabetes Metab Res Rev* 2001; **17**: 412-421 [PMID: 11757076 DOI: 10.1002/dmrr.236]
- 58 **Engerman RL**, Kern TS. Experimental galactosemia produces diabetic-like retinopathy. *Diabetes* 1984; **33**: 97-100 [PMID: 6360771]
- 59 **Clark SJ**, Keenan TD, Fielder HL, Collinson LJ, Holley RJ, Merry CL, van Kuppevelt TH, Day AJ, Bishop PN. Mapping the differential distribution of glycosaminoglycans in the adult human

- retina, choroid, and sclera. *Invest Ophthalmol Vis Sci* 2011; **52**: 6511-6521 [PMID: 21746802 DOI: 10.1167/iovs.11-7909]
- 60 **Cohen MP**, Ciborowski CJ. Presence of glycosaminoglycans in retinal capillary basement membrane. *Biochim Biophys Acta* 1981; **674**: 400-406 [PMID: 7236737]
  - 61 **Bollineni JS**, Alluru I, Reddi AS. Heparan sulfate proteoglycan synthesis and its expression are decreased in the retina of diabetic rats. *Curr Eye Res* 1997; **16**: 127-130 [PMID: 9068943]
  - 62 **Moschos MM**, Rouvas AA, Papadimitriou S, Kotsolis A, Sitaras N, Apostolopoulos M. Quantitative determination of glycosaminoglycans in tears of diabetic patients. *Clin Ophthalmol* 2008; **2**: 581-584 [PMID: 19668757]
  - 63 **Nishiguchi KM**, Kataoka K, Kachi S, Komeima K, Terasaki H. Regulation of pathologic retinal angiogenesis in mice and inhibition of VEGF-VEGFR2 binding by soluble heparan sulfate. *PLoS One* 2010; **5**: e13493 [PMID: 20975989 DOI: 10.1371/journal.pone.0013493]
  - 64 **Murakami M**, Simons M. Fibroblast growth factor regulation of neovascularization. *Curr Opin Hematol* 2008; **15**: 215-220 [PMID: 18391788 DOI: 10.1097/MOH.0b013e3282f97d98]
  - 65 **Yenice O**, Kazokoglu H, Ozcan E, Yüksel M, Adigüzel G, Haklar G, Yavuz DG. Erythrocyte Membrane Anionic Content and Urinary Glycosaminoglycan Excretion in Type 1 Diabetes: Association with Retinopathy. *Curr Eye Res* 2006; **31**: 975-981 [PMID: 17114123 DOI: 10.1080/02713680600991445]
  - 66 **Cechowska-Pasko M**, Pałka J, Bańkowski E. Decrease in the glycosaminoglycan content in the skin of diabetic rats. The role of IGF-I, IGF-binding proteins and proteolytic activity. *Mol Cell Biochem* 1996; **154**: 1-8 [PMID: 8717410]
  - 67 **Taylor WH**, Sinha A, Khan IA, McDaniel ST, Esko JD. Primers of glycosaminoglycan biosynthesis from Peruvian rain forest plants. *J Biol Chem* 1998; **273**: 22260-22266 [PMID: 9712841 DOI: 10.1074/jbc.273.35.22260]
  - 68 **Nandini CD**, Sambaiah K, Salimath PV. Dietary fibres ameliorate decreased synthesis of heparan sulphate in streptozotocin induced diabetic rats. *J Nutr Biochem* 2003; **14**: 203-210 [PMID: 12770644]
  - 69 **Kumar GS**, Shetty AK, Salimath PV. Modulatory effect of bitter melon (*Momordica charantia* LINN.) on alterations in kidney heparan sulfate in streptozotocin-induced diabetic rats. *J Ethnopharmacol* 2008; **115**: 276-283 [PMID: 18024034 DOI: 10.1016/j.jep.2007.10.002]
  - 70 **Joladarashi D**, Chilkunda ND, Salimath PV. Tinospora cordifolia consumption ameliorates changes in kidney chondroitin sulphate/dermatan sulphate in diabetic rats. *J Nutr Sci* 2012; **1**: e7 [PMID: 25191554 DOI: 10.1017/jns.2012.6]
  - 71 **Piotrowska E**, Jakóbkiewicz-Banecka J, Barańska S, Tyłki-Szymańska A, Czartoryska B, Węgrzyn A, Węgrzyn G. Genistein-mediated inhibition of glycosaminoglycan synthesis as a basis for gene expression-targeted isoflavone therapy for mucopolysaccharidoses. *Eur J Hum Genet* 2006; **14**: 846-852 [PMID: 16670689]
  - 72 **Kalea AZ**, Lamari FN, Theocharis AD, Schuschke DA, Karamanos NK, Klimis-Zacas DJ. Dietary manganese affects the concentration, composition and sulfation pattern of heparan sulfate glycosaminoglycans in Sprague-Dawley rat aorta. *Biomaterials* 2006; **19**: 535-546 [PMID: 16937260]
  - 73 **Kalea AZ**, Lamari FN, Theocharis AD, Cordopatis P, Schuschke DA, Karamanos NK, Klimis-Zacas DJ. Wild blueberry (*Vaccinium angustifolium*) consumption affects the composition and structure of glycosaminoglycans in Sprague-Dawley rat aorta. *J Nutr Biochem* 2006; **17**: 109-116 [PMID: 16111874 DOI: 10.1016/j.jnutbio.2005.05.015]
  - 74 **Ponrasu T**, Suguna L. Efficacy of *Annona squamosa* L. in the synthesis of glycosaminoglycans and collagen during wound repair in streptozotocin induced diabetic rats. *Biomed Res Int* 2014; **2014**: 124352 [PMID: 25003104 DOI: 10.1155/2014/124352]
  - 75 **Cilla A**, Olivares M, Laparra JM. Glycosaminoglycans from Animal Tissue Foods and Gut Health. *Food Rev Int* 2013; **29**: 192-200 [DOI: 10.1080/87559129.2012.751546]
  - 76 **Lewis EJ**, Lewis JB, Greene T, Hunsicker LG, Berl T, Pohl MA, de Zeeuw D, Heerspink HL, Rohde RD, Atkins RC, Reutens AT, Packham DK, Raz I. Sulodexide for kidney protection in type 2 diabetes patients with microalbuminuria: a randomized controlled trial. *Am J Kidney Dis* 2011; **58**: 729-736 [PMID: 21872376 DOI: 10.1053/j.ajkd.2011.06.020]
  - 77 **Asari A**, Kanemitsu T, Kurihara H. Oral administration of high molecular weight hyaluronan (900 kDa) controls immune system via Toll-like receptor 4 in the intestinal epithelium. *J Biol Chem* 2010; **285**: 24751-24758 [PMID: 20504769 DOI: 10.1074/jbc.M110.104950]

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Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China  
Telephone: +86-10-85381891

Fax: +86-10-85381893

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## Use of fibrates in the metabolic syndrome: A review

Kate E Shipman, Richard C Strange, Sudarshan Ramachandran

Kate E Shipman, Department of Clinical Biochemistry, University Hospital Birmingham, Birmingham B15 2TH, United Kingdom

Richard C Strange, Institute for Science and Technology in Medicine, Keele University Medical School, Staffordshire ST5 5BG, United Kingdom

Sudarshan Ramachandran, Department of Clinical Biochemistry, Heart of England NHS Foundation Trust, Good Hope Hospital, West Midlands B75 7RR, United Kingdom

Sudarshan Ramachandran, Department of Clinical Biochemistry, University Hospitals of North Midlands and Faculty of Health Sciences, Staffordshire University, West Midlands ST5 5BG, United Kingdom

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**Correspondence to:** Sudarshan Ramachandran, PhD, FRCPATH, Professor, Department of Clinical Biochemistry, Heart of England NHS Foundation Trust, Good Hope Hospital, Rectory Road, Sutton Coldfield, West Midlands B75 7RR, United Kingdom. [sud.ramachandran@heartofengland.nhs.uk](mailto:sud.ramachandran@heartofengland.nhs.uk)  
 Telephone: +44-121-4247246  
 Fax: +44-121-3111800

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### Abstract

The use of fibrates in the treatment of dyslipidaemia has changed significantly over recent years. Their role appeared clear at the start of this century. The Helsinki Heart Study and Veterans Affairs High-Density Cholesterol Intervention Trial suggested significant benefit, especially in patients with atherogenic dyslipidaemia. However, this clarity disintegrated following the negative outcomes reported by the Bezafibrate Infarction Prevention, Fenofibrate Intervention and Event Lowering in Diabetes and Action to Control Cardiovascular Risk in Diabetes randomised controlled trials. In this review we discuss these and other relevant trials and consider patient subgroups such as those with the metabolic syndrome and those needing treatment to prevent the microvascular complications associated with diabetes in whom fibrates may be useful. We also discuss observations from our group that may provide some explanation for the varying outcomes reported in large trials. The actions of fibrates in patients who are also on statins are interesting and appear to differ from those in patients not on statins. Understanding this is key as statins are the primary lipid lowering agents and likely to occupy that position for the foreseeable future. We also present other features of fibrate treatment we have observed in our clinical practice; changes in creatinine, liver function tests and the paradoxical high density lipoprotein reduction. Our purpose is to provide enough data for the reader to make objective decisions in their own clinical practice regarding fibrate use.

**Key words:** Fibrates; Metabolic syndrome; Paradoxical high density lipoprotein cholesterol decrease; High density lipoprotein cholesterol; Cardiovascular disease; Peroxisome proliferator-activated receptor; Randomised control trial; Triglycerides

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**Core tip:** Atherogenic dyslipidaemia is characterised by low high density lipoprotein cholesterol (HDL-C) and raised triglycerides, this pattern being associated with adverse cardiovascular risk. The fibrate class of drugs has been shown to both elevate HDL-C and reduce triglyceride concentrations. Despite several randomised control trials the data remain conflicting in regards to the use of fibrates in cardiovascular disease management. Our objective is to consolidate and summarise the literature to clarify the current evidence base.

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## INTRODUCTION

Cardiovascular disease (CVD) is a leading cause of death worldwide<sup>[1]</sup> and in the United Kingdom in 2012, accounted for 28% of deaths<sup>[2]</sup>. Treatment is based on reducing modifiable risk factors with lowering of serum lipid levels one of the major targets<sup>[3]</sup>. Despite overwhelming evidence showing that statin-induced reduction of serum low density lipoprotein cholesterol (LDL-C) is associated with a marked reduction in CVD risk<sup>[4]</sup> there appears to be a high residual risk<sup>[5]</sup> perhaps due to other lipoprotein particles associated with cardiovascular risk<sup>[6-8]</sup>. Therefore, additional therapies may be useful to target these atherogenic lipoprotein particles and it is in this context that fibrates could have a useful clinical role. The evidence for this claim will be reviewed.

## ATHEROGENIC DYSLIPIDAEMIA, THE METABOLIC SYNDROME AND CARDIOVASCULAR DISEASE

Statins competitively inhibit HMG CoA reductase and thereby effect a decrease in hepatic cholesterol synthesis. This results in the up-regulation of LDL receptors consequently increasing LDL uptake which in turn lowers plasma cholesterol<sup>[9]</sup>. Following the 4S secondary prevention randomised control trial (RCT) in 1994<sup>[10]</sup>, statins have formed the cornerstone of lipid reduction strategy in CVD prevention guidelines<sup>[3,11]</sup>. The "lower is better" hypothesis regarding cholesterol and LDL-C levels<sup>[4]</sup> has been supported by trials including treating to new targets<sup>[12]</sup>, The Reversal of Atherosclerosis with Aggressive Lipid Lowering<sup>[13]</sup> and Pravastatin or Atorvastatin Evaluation and Infection

Therapy<sup>[14]</sup>. Importantly, studies such as A Study to Evaluate the Effect of Rosuvastatin on Intravascular Ultrasound Derived Coronary Atheroma Burden (ASTEROID)<sup>[15]</sup> and Study of Coronary Atheroma by Intravascular Ultrasound: Effect of Rosuvastatin vs Atorvastatin (SATURN)<sup>[16]</sup> suggest even regression of atheromatous plaque is possible if LDL-C levels are lowered sufficiently.

However, despite optimal reduction of LDL-C with statins and, correction of other modifiable risk factors, CVD risk is not eliminated<sup>[6]</sup>. The source of this residual risk may be due to other atherogenic lipid species such as reduced high density lipoprotein cholesterol (HDL-C) and/or raised triglycerides (TG) which are only modestly affected by statin therapy. The association between CVD and low HDL-C was first reported by Barr *et al.*<sup>[17]</sup> nearly 60 years ago and confirmed in prospective studies such as the Framingham Heart Study<sup>[18]</sup> and the Munster Heart Study<sup>[19,20]</sup>. This association appears to be independent of LDL-C<sup>[18]</sup>. Cardiovascular event rates in statin trials also reflect this; when the study cohort is stratified by HDL-C, HDL-C levels remain associated with CVD even following LDL-C reduction<sup>[6]</sup>. Elevated TG levels have also been linked with CVD in studies such as multiple risk factor intervention trial and the Copenhagen City Heart Study<sup>[7,8]</sup>.

The lipid profile characterised by low HDL-C and high TG is termed atherogenic dyslipidaemia or the atherogenic lipoprotein phenotype (Table 1). This forms one of the characteristic features of the metabolic syndrome. This syndrome gained global recognition following the Banting Lecture delivered by Reaven<sup>[21]</sup> in 1988 to the American Diabetes Association. He termed the combination of hypertension, dyslipidaemia and glucose intolerance as syndrome X and suggested that affected individuals were at higher risk of atherosclerosis<sup>[21]</sup>. The International Classification of Disease code now terms syndrome X, the metabolic syndrome<sup>[22]</sup>. Various groups have provided classification systems for the metabolic syndrome (Table 1)<sup>[23]</sup>. These include the World Health Organisation (WHO)<sup>[24]</sup>, European Group for the Study of Insulin Resistance<sup>[25]</sup>, American College of Endocrinology<sup>[26]</sup>, National Cholesterol Education Program - Adult Treatment Panel III<sup>[27]</sup> and, more recently, the international diabetes federation (IDF)<sup>[28]</sup>. Although the classifying characteristics are the same in these classifications, the thresholds for inclusion differ. A consensus was reached in 2009 with the IDF, National Heart, Lung and Blood Institute, American Heart Association, World Heart Federation, International Atherosclerosis and the International Association for the Study of Obesity agreeing on threshold levels that mirrored those of the IDF<sup>[29]</sup>.

There is debate as to the clinical usefulness of making a diagnosis of metabolic syndrome. While the syndrome is associated with a doubling of CVD risk<sup>[30]</sup>, much of this increase is the sum of the individual classifying components<sup>[31]</sup>. Bayturan *et al.*<sup>[32]</sup> reviewed 3459 patients

**Table 1** The thresholds defining the metabolic syndrome issued by individual organisations (reproduced from: Strange *et al*<sup>[23]</sup>)

Group	WHO <sup>[24]</sup>	EGIR <sup>[25]</sup>	NCEP/ATP III <sup>[27]</sup>	AACE <sup>[26]</sup>	IDF consensus <sup>[28]</sup>	IDF consensus (10 to < 16 yr) <sup>[112]</sup>
Definition	IGT, IFG, T2DM or lowered insulin sensitivity plus 2 of the following	Plasma insulin > 75 <sup>th</sup> percentile plus 2 of the following	3 of the following	IGT or IFG plus any of the following based on clinical judgement	See below	
Europoid waist circumference (cm)	W:H > 0.90 M, W:H > 0.85 F or BMI > 30 kg/m <sup>2</sup>	≥ 94 M, ≥ 80 F	≥ 102 M, ≥ 88 F	BMI ≥ 25 kg/m <sup>2</sup>	≥ 94 M, ≥ 80 F or BMI > 30 kg/m <sup>2</sup> plus 2 of the following	> 90 <sup>th</sup> percentile plus 2 of the following
Triglyceride (mmol/L)	> 1.7	> 1.7	≥ 1.7	> 1.7	> 1.7	≥ 1.7
HDL (mmol/L)	< 0.91 M, < 1.01 F	< 0.91	< 1.03 M, < 1.29 F	< 1.03 M, < 1.29 F	< 1.03 M, < 1.29 F	< 1.03
BP (mmHg)	≥ 140/90	≥ 140/90 or on treatment	≥ 130/85	≥ 130/85	SBP ≥ 130 or DBP ≥ 85 or on treatment	SBP ≥ 130 and/or DBP ≥ 85
Glucose (mmol/L)	IGT, IFG or T2DM	IGT or IFG (but not diabetes)	≥ 5.6 <sup>[113]</sup> or diabetes	IGT or IFG (but not diabetes)	≥ 5.6	≥ 5.6 or known T2DM
Others	Microalbuminuria ACR > 30 mg/g			Other features of IR <sup>1</sup>		

<sup>1</sup>Includes polycystic ovary syndrome, family history or ethnic group susceptible to type 2 diabetes, sedentary lifestyle and advancing age. ACR: Albumin creatinine ration; BMI: Body mass index; DBP: Diastolic blood pressure; F: Female; IFG: Impaired fasting glucose; IGT: Impaired glucose tolerance; IR: Insulin resistance; SBP: Systolic blood pressure; M: Male; T2DM: Type 2 diabetes mellitus; W:H: Waist to hip ratio; HDL: High density lipoprotein; WHO: World Health Organisation; NCEP: National cholesterol education program; EGIR: European group for the study of insulin resistance; IDF: International diabetes federation; AACE: American Association of Clinical Endocrinologists.

included in 7 trials using plaque progression measured by intravascular ultrasonography as outcome. Although the metabolic syndrome was significantly associated with increased plaque, the relationship lost significance when adjusted for serum TG, body mass index (BMI), HDL-C, blood pressure value/treatment of hypertension. In the multiple regression model just serum TG concentrations > 1.70 mmol/L remained significantly associated with plaque progression, this perhaps making a further case for fibrate therapy as potent triglyceride reducing agents in hypertriglyceridaemia<sup>[32]</sup>.

Although evidence indicates that the metabolic syndrome does not add prognostic risk in an individual patient, we and others<sup>[33]</sup> argue that it has practical merit. Awareness of the metabolic syndrome makes it easier to adopt a holistic approach to care rather than focussing on individual risk factors. It may be even more useful in a research setting. The characteristics of the syndrome are associated with each other, but also individually increase CVD risk. Thus, the entire network of risk factors must be considered. Indeed, the various phenotypes of the syndrome prompt the use of specific therapies. For example, the syndrome is characterised by weight gain that may be ameliorated by nutritional advice resulting in weight loss and reduced hypertriglyceridaemia and increased HDL. Such advice and intervention should be first line treatment which is continued throughout treatment even if initial efforts are unsuccessful. Insulin resistance and hypertension may also be improved by this approach. Fibrates, by causing elevation of HDL-C and reduction of TG, may be ideally suited to treating people with this condition. The discovery and action of fibrates will now be described.

## DISCOVERY AND DEVELOPMENT OF FIBRATES

Chemically fibrates are based on phenylethyl acetic acid, a derivative of dehydrocholic acid. The compounds were developed as insecticides by imperial chemical industries (ICI) Laboratories in England (the pharmaceutical division is now part of Astra Zeneca) and accidental exposure by farm workers in France in 1953 provided the first observation that they could cause reduction of lipids in serum<sup>[34]</sup>. This finding was confirmed in rats though the mechanism of cholesterol reduction was unknown<sup>[35]</sup>. Subsequently, work at ICI identified several oxyisobutyric acid derivatives with similar effects, an observation that lead in 1962 to the development of clofibrate (ethyl- $\alpha$ -4-chlorophenoxyisobutyrate), the first therapeutically useful fibrate<sup>[36]</sup>.

Fenofibrate, which is a benzoyl derivative of clofibrate with higher efficacy, was produced in 1974<sup>[37]</sup>. In the late 1970's and early 1980's gemfibrozil<sup>[38]</sup>, bezafibrate<sup>[39]</sup> and ciprofibrate<sup>[40]</sup> were added to the structurally diverse fibrate family<sup>[41]</sup>. Clofibrate and fenofibrate are pro-drugs that are hydrolysed to their active metabolites clofibric acid and fenofibric acid respectively, while gemfibrozil and bezafibrate are active compounds. Development of fibrates as a class was hindered by concerns of murine hepatic carcinogenicity thought to be associated with peroxisomal proliferation<sup>[42,43]</sup>. However, Blümcke *et al*<sup>[44]</sup>, in 1983 demonstrated that humans were not at increased risk of hepatic tumour formation. Clofibrate became non-viable as a therapeutic agent when the WHO cooperative trial, a primary prevention trial, showed a 47% increase in mortality, mainly non-cardiac,

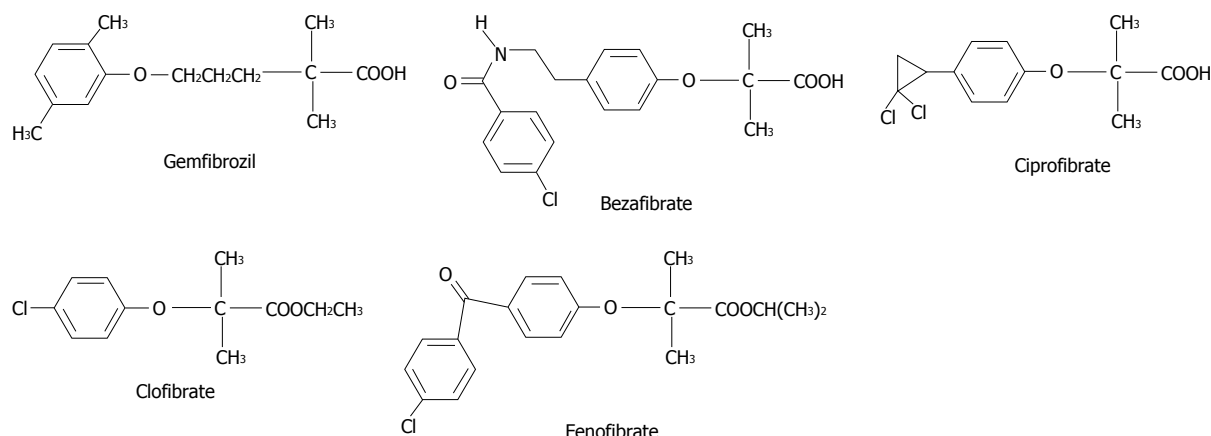


Figure 1 Chemical structures of fibrates that have been used clinically.

in the treatment arm<sup>[45]</sup>. The other fibrates remain clinically available though their role is under review following variable outcomes in RCTs (see below).

## METABOLISM OF FIBRATES

With the exception of gemfibrozil, the role of individual members of the phase 1 detoxifying cytochrome p450 supergene family in the metabolism of fibrates is unclear. Significantly, gemfibrozil inhibits CYP2C8 which is involved in the metabolism of the statin cerivastatin, thereby potentially causing an increase in the plasma concentration of the statin and an increased risk of rhabdomyolysis. Subsequently in 2001, cerivastatin was withdrawn<sup>[46]</sup>. It is recommended that when treating patients with both a fibrate and statin that gemfibrozil be avoided<sup>[47]</sup>. Other fibrates in clinical use have not shown a similar interaction with statins. Fibrates also cause reversible elevations in creatinine levels and, though this is not usually a contraindication for use, fibrate dose should be reduced or the drug withheld in those with renal impairment as recommended by the manufacturer<sup>[47]</sup>. Doses of fibrates should be reduced or withheld in those with renal impairment as per manufacturer's advice. The chemical structures of fibrates in clinical use are shown in Figure 1.

## FIBRATE PHARMACOLOGY

Initially, fibrates were thought to work *via* an androsterone-like effect<sup>[36]</sup> though later it was realised that their therapeutic target was the nuclear peroxisome proliferator-activated receptor (PPAR). Nuclear receptors are one type of receptor capable of recognising external stimuli and effecting internal changes *via* mediation of expression of key genes and hence, protein synthesis. In the 1980's it was recognised that fibrates affect transcription of various proteins associated with lipid metabolism<sup>[48,49]</sup> and it is now known that PPAR receptors are one of the cell's mechanisms for regulation of energy homeostasis.

PPAR $\alpha$  was first cloned in the mouse<sup>[50]</sup>, and this was followed 2 years later by work from Dreyer *et al.*<sup>[51]</sup> who cloned 3 types of PPAR (PPAR $\alpha$ , PPAR $\gamma$  and PPAR $\beta/\delta$ ) in *Xenopus*. PPAR $\alpha$  has subsequently been identified in other species (*e.g.*, humans, amphibians, teleosts and cyclostomes)<sup>[52]</sup>. There are structural similarities between the 3 PPAR subtypes. LDL and very LDL (VLDL) activate PPAR $\alpha$  in the presence of lipoprotein lipase which suggests that esterified triacylglycerols and fatty acids may be the natural ligands<sup>[53]</sup>.

The PPAR-retinoid X receptor (RXR) heterodimer exists in active and inactive states. When inactive it is bound to co-repressors such as the nuclear receptor co-repressor or the silencing mediator for retinoid and thyroid hormone receptors. When a ligand binds to either PPAR or RXR a conformational change in the heterodimer takes place and the co-repressor dissociates in order for the complex to bind and activate co-activators such as the steroid receptor co-activator 1. When the PPAR-RXR complex is activated it binds to the peroxisome proliferator response element found in the upstream region of target genes<sup>[54]</sup> and induces transcription.

Fibrates bind and activate PPAR $\alpha$  and regulate gene expression, thereby influencing fatty acid and lipoprotein metabolism in liver, muscle, both skeletal and cardiac, and kidney<sup>[55]</sup>. Synthesis of apoprotein (Apo)-A I, Apo-A II, ApoC-III, lipoprotein lipase, ATP-binding cassette transporter A1, cholesterol ester transfer protein, scavenger receptor class B-type 1 and ApoA5<sup>[56-58]</sup>, factors mainly controlling HDL and VLDL metabolism, are altered by activated PPAR $\alpha$ . Thus, fibrate therapy leads to increased HDL-C concentrations, a greater reduction in TG levels and a modest decrease in LDL-C concentrations<sup>[59]</sup>. Fibrates also have a role in preventing the hypertriglyceridaemia associated with pancreatitis. Guidelines do suggest fibrates as first-line TG lowering treatment to reduce the risk of pancreatitis, with nicotinic acid, omega 3 supplements and statins also considered<sup>[60]</sup>.

The clinical efficacy of fibrates in CVD risk management will now be examined.



**Table 2** Details of the large fibrate outcome trials in the total cohort are provided in this table

Trial	HHS	VA-HIT	BIP	FIELD	ACCORD
Drug	Gemfibrozil	Gemfibrozil	Bezafibrate	Fenofibrate	Fenofibrate
Dose	1200 mg/d	1200 mg/d	400 mg/d	200 mg/d	200 mg/d
Primary endpoint	MI (fatal and non-fatal), cardiac death	Combined incidence of nonfatal MI and death from CAD	MI (fatal and non-fatal), sudden death	CHD death, non-fatal MI	Non-fatal MI, non-fatal stroke, or CVD death
Mean follow-up (yr)	5	5	6	5	5
Patients (total)	Fibrate = 2051; placebo = 2030	Fibrate = 1264; placebo = 1267	Fibrate = 1548; placebo = 1542	Fibrate = 4895; placebo = 4900	Fibrate = 2765; placebo = 2753
Effect on Lipids (%)	LDL-C: -10; TC: -11; TG: -43; HDL-C: +10	LDL-C: 0; TC: -4; TG: -31; HDL-C: +6	LDL-C: -6.5; TC: -4.5; TG: -21; HDL-C: +18	LDL-C: -12; TC: -11; TG: -29; HDL-C: +5	LDL-C: -19; TC: -14; TG: -22; HDL-C: +8.4
Outcomes	CHD: ↓ 34%; non-fatal MI: ↓ 37%; total mortality: No change	CHD and non-fatal MI: ↓ 22%; total mortality: ↓ 11% (NS)	Fatal and nonfatal MI and sudden death: ↓ 9% (NS); total mortality: No change	CHD and non-fatal MI: ↓ 11% (NS); total mortality: ↑ 19% (NS)	Nonfatal MI nonfatal stroke CVD death: ↓ 8% (NS); total mortality: ↓ 9% (NS)

HHS: Helsinki Heart Study; VA-HIT: Veterans Affairs High-Density Cholesterol Intervention Trial; BIP: Bezafibrate Infarction Prevention; FIELD: Fenofibrate Intervention and Event Lowering in Diabetes; ACCORD: Action to Control Cardiovascular Risk in Diabetes; CVD: Cardiovascular disease; NS: Not significant; LDL-C: Low density lipoprotein cholesterol; TC: Total cholesterol; TG: Triglycerides; HDL-C: High density lipoprotein cholesterol; MI: Myocardial infarction; CAD: Coronary artery disease; CHD: Coronary heart disease.

## FIBRATE INTERVENTION TRIALS: GEMFIBROZIL

There is a theoretical basis behind the hypothesis that fibrates would be a useful adjunct in CVD risk management; epidemiological studies indicate their value in the treatment of the atherogenic dyslipidaemia associated with CVD risk. However, published data are conflicting. RCTs with gemfibrozil<sup>[61,62]</sup> have shown overall benefit while those using fenofibrate<sup>[63,64]</sup> and bezafibrate<sup>[65]</sup> have been negative in terms of the primary end point. Details of these trials including the primary end points are presented in Table 2. Clearly, these data are very different to those obtained from trials using statins; all members of that drug group demonstrate CVD risk-lowering effects. The larger fibrate RCTs will be described and reasons suggested for these discrepancies.

Considering the positive gemfibrozil studies first, the helsinki heart study (HHS) lasted for 5 years and studied a large cohort of men comparing 600 mg gemfibrozil twice daily against placebo (2051 and 2030 men respectively). Inclusion criteria included adult men aged 40-55 years with a non-HDL-C > 5.2 mmol/L<sup>[61]</sup>. The mean LDL-C of the study group was 4.88 mmol/L<sup>[66]</sup>. The comparison of relative risk in cardiovascular outcomes demonstrated a 34% statistically significant reduction with fibrate therapy; 27.3 events in the gemfibrozil and 41.4 events in the placebo group per 1000 individuals<sup>[61]</sup>. Prior to the widespread use of statins this was a simple comparison of fibrates against no other lipid lowering therapy. Subgroup analysis demonstrated that those with an elevated TG and reduced HDL-C level had greater relative risk reduction providing the first evidence that fibrates may be most useful in those with atherogenic dyslipidaemia<sup>[67]</sup>.

The second large, gemfibrozil study (2531 participants), the Veterans Affairs High-Density Cholesterol Intervention Trial (VA-HIT), selected patients with

established coronary disease<sup>[62]</sup>. Completed over 10 years after the HHS this again compared gemfibrozil (1200 mg/d) vs placebo and only in men. Inclusion age was higher, selecting those aged < 74 years, and low HDL-C < 1.0 mmol/L. LDL-C was not affected by treatment, but HDL-C was elevated by 6% and TGs decreased by 31% and total cholesterol (TC) by 4% when compared to placebo. The primary outcome in this secondary prevention RCT was myocardial infarction or cardiovascular death. It is also worth noting that the LDL-C of the cohort was below 3.6 mmol/L, in an attempt to negate LDL-C related CVD risk. The median follow-up was 5.1 years and during this period the relative risk was significantly reduced by 22% in the treatment group (absolute event rate was 17.3% in the treatment vs 21.7% in the placebo control arms), and even more so in individuals with features of insulin resistance when a sub group analysis was carried out<sup>[62]</sup>.

## FIBRATE INTERVENTION TRIALS: FENOFIBRATE

The clinical usefulness of Fenofibrate has been assessed in 2 large RCTs; the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) and Action to Control Cardiovascular Risk in Diabetes (ACCORD-LIPID) trials<sup>[63,64]</sup>. The FIELD trial was the larger and selected only patients with type 2 diabetes mellitus. In total, 9795 participants (4895 given fenofibrate and 4900 placebo) aged between 50-75 years were enrolled and followed up for a mean of 5 years; 2131 participants had a previous history of CVD. Unlike VA-HIT, which used low HDL-C as an inclusion criterion<sup>[62]</sup>, TG were the primary lipid type with either the TG/HDL-C ratio ( $\geq 4.0$ ) or TG (1.0-5.9 mmol/L) level defining inclusion. In addition TC had to be between 3.9-6.5 mmol/L and those with plasma creatinine > 130  $\mu$ mol/L were excluded. A non-significant difference in coronary events, the primary outcome was observed; 5.2% participants in

the fenofibrate arm and 5.9% in the control arm<sup>[63]</sup>.

Interpretation of these data are complicated by the addition of statin therapy during the follow-up to any individual not satisfying the TC and LDL-C targets due to the evidence based change in practice at the time (2002 - publication of the heart protection study)<sup>[68]</sup>. This resulted in different rates of statin treatment; 17% in the placebo and 8% in the fenofibrate arms. This discrepancy could have masked any benefits gained from fenofibrate therapy and, effected changes in the lipid profile. In the fenofibrate treatment group reductions of TC by 11%, LDL-C by 12%, and TG by 29%, with an increase in HDL-C of 5% after 4 mo, were observed (the improvement in HDL-C dropped from 5% to 2% at the end of the 5 years, the reasons for this are unclear)<sup>[63]</sup>.

In a *post hoc* examination of the FIELD study data, 80% of the cohort met the criteria for metabolic syndrome<sup>[27,69]</sup>. CVD events were significantly reduced in those with low HDL-C, hypertension and most of all in those with marked atherogenic dyslipidemia (27% relative risk reduction, number needed to treat to prevent one coronary event was 23)<sup>[69]</sup>.

Similar to the FIELD study, the ACCORD-LIPID also examined the effects of fenofibrate in those with type 2 diabetes mellitus and an HbA1c  $\geq 7.5\%$  (58 mmol/mol). Published 5 years (2010) after the FIELD study, statin therapy based on LDL-C targets was now well established and therefore, the study was designed to examine whether additional benefit could be gained from adding fenofibrate to simvastatin therapy. With fewer patients than FIELD, 5518 patients, on open-label simvastatin with or without established CVD, were randomised to fenofibrate (2765) or placebo (2753). Age criteria was 40-79 years for those with CVD and increased to 55-79 years for those with 2 additional CVD risk factors followed up for a mean of 4.7 years. When glycated haemoglobin was  $\geq 58$  mmol/mol together with established CVD the age at entry was restricted to 40-79 years<sup>[64]</sup>.

The range of LDL-C was 1.55-4.65 mmol/L and HDL-C was  $< 1.42$  mmol/L for women and  $< 1.29$  mmol/L for men; TGs were  $< 8.5$  mmol/L for those in the treatment arm and  $< 4.5$  mmol/L for those on placebo. LDL-C reduction was similar in the treatment and placebo arms but HDL-C was significantly increased, albeit only to a small degree (0.09 mmol/L vs 0.06 mmol/L), and TGs significantly lower (0.47 mmol/L vs 0.18 mmol/L) in the treatment arm. First occurrence of the primary outcome, non-fatal myocardial infarction or stroke or death from cardiovascular causes, was not significantly different being 2.2% in the treatment arm compared with 2.4% in the placebo. Secondary outcomes, individual components of the primary outcomes and a composite of the primary outcomes including total mortality, revascularisation and admission to hospital for heart failure, were also not significantly different<sup>[64]</sup>.

The diabetes atherosclerosis intervention study (DAIS), non-clinical endpoint trial, coronary artery athero-

sclerosis progression on angiogram, with fenofibrate looked at 418 men and women (207 and 211 subjects in the fenofibrate group and placebo groups respectively) with type 2 diabetes (mean HbA1c: 48 mmol/mol) with at least one demonstrable coronary lesion. This small trial did show a significantly slower rate of atherosclerosis progression but was not powered towards clinical outcomes<sup>[70]</sup>. Davidson *et al.*<sup>[71]</sup>, in the Fenofibrate Reducing Residual Risk on Statin Therapy trial, also examined a non-clinical endpoint, carotid intimal thickening, in participants on atorvastatin with 342 randomised to placebo and 340 patients to fenofibrate. Inclusion criteria included a dyslipidaemia and history of vascular disease. Unlike DAIS, no significant difference was found in endpoint between the treatment groups<sup>[71]</sup>.

## FIBRATE INTERVENTION TRIALS:

### BEZAFIBRATE

A large bezafibrate RCT, the Bezafibrate Infarction Prevention (BIP) study, studied a secondary prevention population, previous myocardial infarction or angina, over 6.2 years. A total of 3090 men and women aged 45-74 years were randomised to bezafibrate 400 mg vs placebo with starting TC 4.7-6.5 mmol/L and HDL-C  $\leq 1.17$  mmol/L, TG  $\leq 3.4$  mmol/L, and LDL-C  $\leq 4.65$  mmol/L. Non-significant reductions in myocardial infarctions (both fatal and non-fatal) and sudden death occurred in the treatment arm (13.6%) vs placebo (15%). Treatment did however, increase HDL-C by 18% and reduce TG by 21%<sup>[65]</sup>.

Subgroup analysis of the BIP study data demonstrated a significant and large reduction (almost 40%) in the primary end point, reduction of fatal or non-fatal myocardial infarction or sudden death, in those with TG  $\geq 2.26$  mmol/L<sup>[65]</sup>. When longer term data and subgroup analysis used in a *post hoc* analysis focused on those with 3 of the 5 risk factors classifying the metabolic syndrome (HDL-C, TG, glucose, BMI and blood pressure), the myocardial infarction and non-fatal myocardial infarction rates were significantly lower as was cardiac mortality<sup>[72]</sup>.

In contrast, a smaller double blinded placebo control trial, Bezafibrate Coronary Atherosclerosis Intervention Trial, besides showing significant increases in HDL-C and decreases in TC, TG, VLDL and fibrinogen, did show significant reduction in coronary event rate. This was mirrored by angiographic improvements assessed by mean minimum lumen diameter. This study selected only males under 45 years of age with dyslipidaemia who had survived a myocardial infarction<sup>[73]</sup>.

Meade *et al.*<sup>[74]</sup> in 2002 recruited 1568 patients with lower extremity arterial disease; 783 of these patients were randomised to bezafibrate. The primary outcome measures (coronary heart disease and stroke), as in the BIP trial, did not show a significant change. The secondary outcomes, such as non-fatal coronary events, were significantly reduced. Further, there was

possible reduction in the severity of claudication. The median value of HDL-C of the study group was relatively high ( $> 1.1$  mmol/L), with median TC of 5.6 mmol/L and median TG between 2.1-2.2 mmol/L in placebo and treatment arms. It may be thought that patients with such values would be better treated with statins, even at the point of recruitment into the study. We do concur with this view. Interestingly significantly more participants in the placebo group dropped out due to being started on a statin than those in the treatment arm<sup>[74]</sup>. This open label nature of the study design leading to unequal treatment with statins between the treatment and control groups, like in the FIELD study, could have influenced the outcome.

Thus, we have several large non-gemfibrozil trials that did not show benefit. Many authors have noted discrepancy between study outcomes, procedures and populations. The open label regarding statin addition (proportions of patients on statins were different between the arms) in the FIELD study and the complicated inclusion criteria seen in the ACCORD-LIPID trial make it difficult to draw conclusions with certainty. The crucial question as to whether fibrates have any role in CVD management remains unanswered except, perhaps in those with marked hypertriglyceridaemia. Sub-group analysis suggests a role for fibrates, but is complicated by lack of study power to detect multiplicity and heterogeneity of treatment effects therefore must be cautiously weighed against<sup>[75]</sup>.

## META-ANALYSIS OF FIBRATE TRIALS

Subgroup analysis suggests a benefit from using fibrates in those with the metabolic syndrome<sup>[67,69,72,76]</sup>. It will, however, need another RCT with narrower inclusion criteria to confirm this benefit. Could this benefit be confirmed by meta-analysis of the existing data? One such study of 5 large trials saw a significant reduction of 28% in cardiovascular risk in those with atherogenic dyslipidaemia, compared with 6% (non-significant) in the comparator group. The study selected only those with HDL-C  $< 0.91$  mmol/L and TG  $> 2.2$  mmol/L, approximately 11%-33% of the trial participants in the 5 trials<sup>[77]</sup>.

A larger meta-analysis, published following the ACCORD-LIPID study<sup>[64]</sup>, summarised 40 years of fibrate outcome data<sup>[78]</sup>. Major cardiovascular events (myocardial infarction and cerebrovascular events), coronary events, coronary revascularisation, stroke, heart failure, cardiovascular deaths and new-onset albuminuria were the outcomes together with side effects. Studies were identified from Medline, Embase and the Cochrane Library database and filtered down to 18 trials comprising 45058 individuals. Mortality from any cause was not significantly reduced but major cardiovascular events were, the greatest effect occurring in trials with higher mean TG levels. There did not appear to be any difference between trials with regards to baseline HDL-C concentrations, type of fibrate or dose used in the

trials<sup>[78]</sup>.

## Other potential factors effecting fibrate efficacy

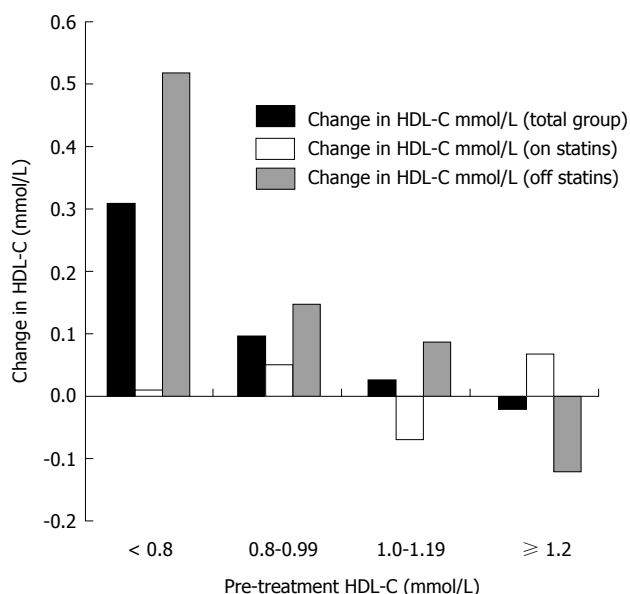
Interestingly there was concern about excess risk in women for the primary outcome (first occurrence of non-fatal myocardial infarction, stroke or death from cardiovascular causes) raised by the ACCORD-LIPID study<sup>[64]</sup>. Sub-group analysis, of the FIELD data however, did not confirm excess risk in women given fenofibrate, instead it suggested that the fibrate may be more efficacious in women compared to men<sup>[79]</sup>.

There is also conflict with regard to which patients gain most benefit from fibrate use: Patients treated for primary or secondary prevention, specific age groups and co morbidities (e.g., diabetes). In sub-group analysis of the HHS data, the 628 participants with suspected coronary heart disease who were excluded from the original study<sup>[61]</sup>, showed no benefit from gemfibrozil, this finding at odds with that from the main study group<sup>[80]</sup>. However this finding was compatible with the findings of the BIP trial of bezafibrate<sup>[65]</sup>. Using ECG data in the FIELD cohort, sub-group analysis revealed that those who had had a silent myocardial infarct gained substantially more benefit from fenofibrate than those who had not with regards future cardiovascular events<sup>[81]</sup>. In a study of individuals with peripheral vascular disease (LEADER study), sub-group analysis suggested that the younger the patient ( $< 65$  years) the more benefit (reduced coronary events) from bezafibrate<sup>[74]</sup>. Those with diabetes appear to have more favourable outcomes from fibrate treatment<sup>[61-63,70]</sup>. However, other studies have not confirmed these findings<sup>[64,71]</sup> though LDL-C levels varied between the studies.

It has also been suggested that those with a higher baseline LDL-C may benefit from HDL elevating therapies, such as fibrates, more than those with a lower starting level<sup>[82]</sup>. In a meta-analysis of therapies (including fibrates) that reduced non-HDL-C and raised HDL-C, non-fatal myocardial infarction occurrence was reduced significantly by treatment, but, when studies that included those with low LDL-C were examined, the reduction was only significant in those with high LDL-C at baseline. Kuhnast *et al.*<sup>[82]</sup> went on to demonstrate that the reduction in non-fatal myocardial infarction appeared to be associated with reduction in LDL-C and was not associated with any elevation in HDL-C<sup>[82]</sup>. This raises the question, if LDL-C levels are reduced sufficiently is there a requirement for additional lipid lowering therapy? However, as we have stated previously, residual risk following LDL-C reduction appears high in patients with low HDL-C. The ACCORD-LIPID trial suggests that this risk may not be reduced by fibrates<sup>[64]</sup>. An understanding of the effect on lipids, of statin/fibrate combination therapy is essential before reaching conclusions. Our work described later will suggest some interesting patterns that need further study.

## Effects of fibrates on microvascular complications

The sequelae of atherosclerotic disease include both



**Figure 2** Change in high-density lipoprotein cholesterol following fibrate treatment stratified by pre-treatment high-density lipoprotein cholesterol concentrations in 248 patients (adapted from Ramachandran *et al.*<sup>[86]</sup>) - permission received via Rightslink. HDL-C: High density lipoprotein cholesterol.

macrovascular and microvascular damage. So far, we have considered the evidence for macrovascular effects though there are data to suggest that fibrates may affect microvascular outcomes. The first study that demonstrated microvascular benefit was the FIELD study which demonstrated a significant reduction in the rate of progression of albuminuria. In those on fenofibrate, the albuminuria improved<sup>[63]</sup>. Creatinine levels were also lower in the original treatment group, when off treatment in a wash-out study 5 years later. The microvascular benefit was maintained with less deterioration and progression of albuminuria with improvement continuing in some<sup>[83]</sup>. Microalbuminuria and macroalbuminuria development were also significantly reduced by fenofibrate in ACCORD-LIPID trial in line with the FIELD study data<sup>[64]</sup>.

Microvascular eye complications have also been studied; in the FIELD study the number of participants requiring laser application for proliferative retinopathy or macular oedema was recorded. There was a significant decrease in laser therapy for retinopathy in those treated with fenofibrate<sup>[84]</sup>. The ACCORD-Eye study looked at secondary outcome data to study factors that might reduce the progression of diabetic retinopathy<sup>[85]</sup>. This analysis demonstrated that fenofibrate in addition to simvastatin and intensive glycaemic control, but not intensive blood pressure control, were significantly effective<sup>[85]</sup>. Therefore, there are data indicating microvascular benefit from fibrates though studies designed to examine microvascular complications as primary endpoints would be beneficial in helping characterise the patient groups to whom benefit would apply.

Thus far we have seen no clear and irrefutable evidence for fibrate use, particular for CVD. There is

however, a tantalising suggestion that treatment of patient subgroups such as those with atherogenic dyslipidaemia and other vascular disease complications including nephropathy and retinopathy, may benefit from treatment with fibrates. The role of fibrates in a post-statin world with a plethora of other CVD drugs is unclear. Though lacking the clarity of double-blinded RCTs there is a wealth of experience of fibrate use and such data can add weight to current theories and identify areas that should be studied in the future. We now describe some observations made by our group that may help explain the current confusion. We will also identify potential roles for fibrates and also highlight suggested work that is required in the future.

## OBSERVATIONS MADE BY OUR RESEARCH GROUP

By 2006, with the publication of the BIP and FIELD studies it became clear that fibrate treatment was associated with inconsistent results. Hence, we established a programme to investigate the metabolic effects of fibrates in an out-patient setting (with about 7000 clinic visits per annum) at the Heart of England Foundation NHS Trust. In addition to changes in lipids we collected data on liver and renal function. We also have collected data on the rare paradoxical change in HDL-C that is seen following fibrates and thiazolidinedione (PPAR $\gamma$  agonists) treatment.

As shown above, analysis of the large RCTs has suggested possible subgroup benefit from fibrate use in those with low pre-treatment HDL-C levels and elevated TG. Our work would support this with HDL-C increase and TG reduction associated independently with lower HDL-C level and higher TG pre-treatment levels respectively<sup>[86,87]</sup>. A significantly greater increase was seen in HDL in this cohort when comparing participants with entry HDL-C < 1.0 mmol/L vs  $\geq$  1.0 mmol/L, but not in those also on statins. The association between HDL-C change and baseline HDL-C (stratified) following fibrates in 257 patients is seen in Figure 2. It is clear that the association is evident only in patients not on statin treatment. These data could support a recommendation that fibrates be reserved for those with low HDL-C<sup>[86]</sup>. Similarly HDL-C levels, after 6 mo of fenofibrate treatment, were also described in 1994 as being greatest in patients with lower pre-treatment HDL-C, although the nature of the association was not well characterised. Mean increase, in 1334 patients, of HDL-C was significant in the total cohort but larger in those with a baseline HDL-C  $\leq$  0.91 mmol/L; 15.2% vs 37.9%<sup>[88]</sup>. Our data suggest that those with high TG may also gain most benefit in TG reduction. This relationship between baseline TG and TG change was not affected by statin treatment. Our series also suggested that TG reduction, while associated with baseline TG levels, was independent of the baseline HDL-C<sup>[87]</sup>.

Both the above findings support the subgroup



analyses from the RCTs; maximum CVD risk reduction being observed in patients with low HDL-C and/or elevated TG levels. However, our data indicates that concurrent statin treatment may lead to a more complex pattern. Our results suggest that the specific benefit from fibrates in patients also treated with statins may lie with TG reduction and not an increase in HDL-C as both changes appear to be independent<sup>[87]</sup>. Our finding is complemented by a recent retrospective study by Scholz *et al.*<sup>[89]</sup> which highlighted the risk posed by elevated TG levels in the metabolic syndrome and pointed to TG lowering by fibrates and omega 3 fatty acids being potentially an important mechanism of cardiovascular event reduction.

The loss of the above association between baseline HDL-C and HDL-C increase (no significant change in HDL-C was observed following fibrates in patients also on statins) in the statin treatment group may be significant in explaining the outcome of the FIELD<sup>[86]</sup>. As we have mentioned previously in the FIELD study more individuals in the placebo group were on statins<sup>[63]</sup>. It is interesting to speculate whether the actions of fibrates are altered by statins. This could also have a bearing on the outcome seen in the ACCORD-LIPID trial<sup>[64]</sup>. Over the past 20 years statins have been the principal lipid lowering agent for CVD risk reduction<sup>[3,11]</sup>. It is extremely unlikely therefore, that fibrates would form the first line intervention in any future CVD guidance. Thus, it is worth further investigating the effects of fibrates on lipids when combined with a statin. The ACCORD-LIPID<sup>[64]</sup> trial did this, but we suggest that the group of patients with the atherogenic lipoprotein pattern be further investigated regarding lipid and lipoprotein changes.

A measure of HDL function/cholesterol efflux as opposed to HDL-C change in various patient subgroups including those also on statins would also be useful in view of the findings of Rohatgi *et al.*<sup>[90]</sup>. When they analysed the data from the Dallas Heart Study it was seen that the relationship between baseline HDL-C and atherosclerotic CVD was not significant [hazard ratio (HR) = 1.08, 95%CI: 0.59-1.99] when adjusted for other traditional risk factors (age, gender, race, diabetes, hypertension, smoking status, BMI, TC, TG and statin use) and HDL particle concentration after a median follow-up of 0.4 years. However, in a similar model, adjusted for the same variables and HDL-C the highest quartile of cholesterol efflux capacity was significantly associated with CVD in comparison with the lowest quartile (HR = 0.33, 95%CI: 0.19-0.55)<sup>[91]</sup>. Thus, we suggest that another dimension has to be added when fibrates are investigated in the future, HDL function should also be looked at in addition to concentration.

Our work examining the role of fibrates in those with conditions related to the atherogenic dyslipidaemia or metabolic syndrome, such as in the treatment of non-alcoholic steatohepatitis (NASH) or non-alcoholic fatty liver disease (NAFLD), is suggestive of benefit. Not only have liver function tests (LFTs) been improved by fibrate use in those with probable NAFLD/NASH but the

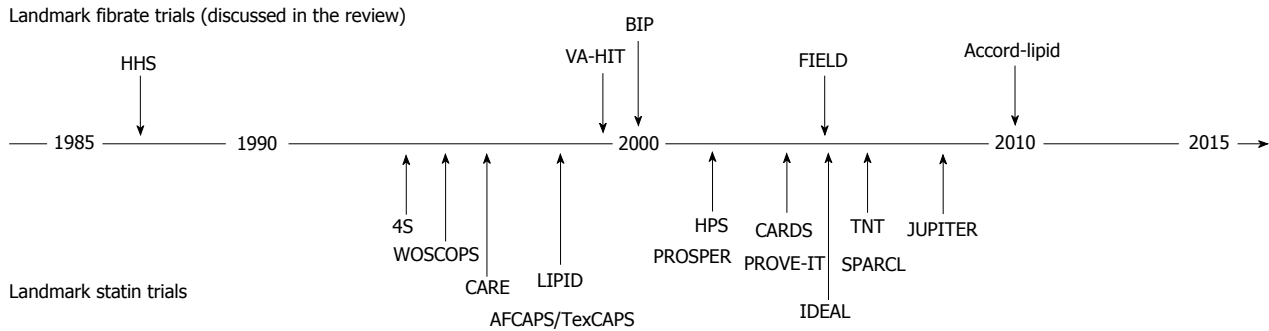
improvements were related to baseline LFTs; greatest benefit gained by those with highest baseline LFTs. It is possible that this reduction was due to treatment of NAFLD/NASH by the fibrate<sup>[92]</sup> which would be in keeping with current theories on NAFLD/NASH aetiology. A two or three hit hypothesis for NAFLD/NASH was initially proposed with accumulation of hepatic fat being the "first hit"<sup>[93,94]</sup>. Oxidative stress, mitochondrial abnormalities and hormonal imbalance (*e.g.*, adiponectin and leptin levels) impairing hepatocyte regeneration and proliferation were considered as possible candidates for the second and third hit<sup>[95,96]</sup>. Fibrates may have a beneficial effect on fatty acid oxidation (reducing hepatic fat accumulation) and inflammation<sup>[95,96]</sup>. Thus, improvement in LFTs associated with fibrates may be due to improving these risk factors.

Our work has also confirmed that increases in creatinine concentration is often seen following fibrate use, in line with that described in the FIELD study<sup>[63,83]</sup>, this increase appears to be reversible on discontinuing treatment. We have described the frequency distribution in estimated glomerular filtration rate (eGFR) that may be seen<sup>[97]</sup>. Clinically this is important as a lower eGFR could lead to withdrawal of other drugs such as metformin, incretins and sodium glucose transporter 2 inhibitors in patients with type 2 diabetes. We also identified the patient group where hypercreatininaemia was more likely: Male gender, lower baseline creatinine concentrations, non-diabetics and greater decrease in TG levels<sup>[98]</sup>.

## PARADOXICAL EFFECTS OF FIBRATES

Despite the evidence clearly demonstrating increases in HDL-C caused by statins there have been "paradoxical HDL-C decreases" described. This rare phenomenon also appears to occur in some treated with either a fibrate or a thiazolidinedione (PPAR $\gamma$  agonists). The paradoxical HDL-C decrease was initially reported by Chandler *et al.*<sup>[99]</sup> in 1994; HDL-C was seen to decrease from 0.9 to 0.18 mmol/L following ciprofibrate treatment. A similar paradoxical response in 2 patients was noted when rosiglitazone was added to fenofibrate<sup>[100]</sup>. In a case series of this phenomenon in 5 patients, we suggested heterogeneity in response following fibrate and rosiglitazone treatment<sup>[101]</sup>. We also showed that pioglitazone also demonstrated this phenomenon, until then only rosiglitazone had been implicated<sup>[102]</sup>. Fibrates and glitazones combined with fibrates have been estimated to reduce HDL-C by 0.02% and 1.39% respectively<sup>[103]</sup>. The pathophysiology of this is unknown but theories include that this is *via* a PPAR $\alpha$  based mechanism as HDL-C metabolism is affected by PPAR $\gamma$  activators *via* the PPAR $\alpha$  receptor<sup>[104]</sup>.

We investigated this rare phenomenon in 25 patients attending our metabolic clinic; the paradoxical HDL-C decrease was defined by us as a reduction in HDL-C of greater than 50%. This relatively large group of patients with this rare phenomenon enabled us to investigate the



**Figure 3 A time line with landmark statin and fibrate randomised control trials (arrows indicate the year of main publication).** The fibrate trials are discussed in the review, while the statin trials are only mentioned when relevant. All the statin trials showed that lowering of LDL-C significantly reduced the cardiovascular event chosen as study outcome. 4S: The scandinavian simvastatin survival study; WOSCOPS: West of scotland coronary prevention study; CARE: Cholesterol and recurrent events trial; LIPID: Long-term intervention with pravastatin in ischaemic disease trial; AFCAPS/TexCAPS: Air force/Texas coronary prevention study; HPS: Heart protection study; PROSPER: The prospective study of pravastatin in the elderly at risk; CARDS: Collaborative atorvastatin diabetes study; PROVE-IT: The pravastatin or atorvastatin evaluation and infection therapy trial; IDEAL: The incremental decrease in events through aggressive lipid lowering study; TNT: Treating to new targets trial; SPARCL: The stroke prevention by aggressive reduction in cholesterol levels trial; JUPITER: Justification for the use of statins in primary prevention: An intervention trial evaluating rosuvastatin trial.

differing presentation patterns<sup>[101,102]</sup>. These included: (1) the phenomenon observed with all fibrates; (2) effect observed with one fibrate, but not other fibrates; (3) when rosiglitazone (but not pioglitazone) was added to a fibrate; (4) when either rosiglitazone or pioglitazone were added to a fibrate; (5) effect only seen when the dose of fibrates and/or glitazones was increased; and (6) decrease in Apo-A1 - seen in all, except in one patient.

Many questions remain about this interesting group of patients. Most of these, such as whether the CVD risk of these patients is different to those not showing this phenomenon, may remain unanswered due to small numbers. Understanding the mechanisms is problematic as clinical heterogeneity is evident even within this rare group. At this stage we would recommend withdrawal of the offending fibrate, although it is worth trying another fibrate, if fibrate treatment is indicated, albeit with regular measures of HDL-C.

## FIBRATES AND CVD: GUIDELINES

The National Institute for Health and Care Excellence (NICE) lipid modification guidelines (CG181) issued in the United Kingdom in 2014 do not recommend routine use of fibrates for primary or secondary prevention, in those with chronic kidney disease or type 1 diabetes<sup>[3]</sup>. Fibrates may be considered in the context of mixed dyslipidaemia and hypertriglyceridaemia though guidance is not particularly specific, particularly for those with TG between 4.5-9.9 mmol/L. For hypertriglyceridaemia urgent specialist referral, for those with TG > 20 mmol/L in the absence of poorly controlled diabetes and alcohol excess, is recommended. Intermediate TG levels (10-20 mmol/L) should trigger a repeat for confirmation and if patients demonstrate a fasting TG > 10 mmol/L they should also be referred to a specialist. The complexity and variety of various dyslipidaemias is not catered for in this guidance and therefore, we urge clinicians to consider individualised care or specialist

referral to make a decision based on the available evidence, pathophysiology and clinical context, which is acknowledged by NICE and recommended in European guidance<sup>[3,11]</sup>. Therefore fibrates are not currently a main feature of CVD risk management. However we urge clinicians to consider them, particularly in those who may gain the most benefit, *e.g.*, atherogenic dyslipidaemia.

## FIBRATES AND CVD: A PERSONAL VIEW

We have well over 20 years of experience in secondary care clinics using all available lipid lowering agents. Our approach to CVD risk reduction is to critically evaluate current evidence and apply it in patient management together with an understanding of the physiology and pathology of atherogenesis. This may be at odds with the current trend which may have led to the NICE guidelines of 2014<sup>[3]</sup>. At present RCTs dominate the landscape. However, RCTs are selective and real world experience should also play a significant part. We do concur that in most cases of dyslipidaemia statins are the front-line agents. Figure 3 shows a time line with landmark statin and fibrate studies highlighted. The statin trials have all showed significant reductions in the cardiovascular events chosen as outcomes in the respective studies. Each successive trial has added another layer of knowledge to the point that further large scale trials may not be of use. As we have seen previously the understanding of fibrates has travelled in the opposite direction. However, after having considered the overall evidence, our view is that fibrates neatly complement statins (and other LDL-C reducing agents). Their action is on VLDL and HDL, particles which are not significantly affected by statins. Fibrates are very complex compounds with a myriad of actions. At times paradoxical effects are seen which add to the complexity.

We have to reiterate that guidelines are for guidance and education. As practitioners of medicine we have

to combine them with knowledge and understanding of the underlying disorder(s). This is the approach we have adopted and we do use fibrates in patients with hypertriglyceridaemia, the decision reached on a case by case basis. Thus, it was reassuring that the subgroup (metabolic syndrome) outcomes and meta-analyses support our viewpoint. Regarding HDL-C we are not certain as to the role of fibrates. This is mainly as HDL metabolism/cholesterol efflux is not measured as opposed to HDL-C. Our approach will change in the future with further study.

## FUTURE DEVELOPMENTS

Other existing TG medications such as omega-3 fatty acids have a less clear evidence base than fibrates in regards to CVD risk reduction. This may be due to lack of specific efficacy or that the benefit is not mediated directly by TG reduction but by other unidentified mechanisms. Further, research is required to answer these questions and also the effects in combination with fibrates.

Development of novel agents that treat hypertriglyceridaemia (including genetic hypertriglyceridaemic states) with greater efficacy may clarify the role of TG reduction in CVD risk management. For example gene therapy with lipoprotein lipase replacement may well reduce pancreatitis in those with familial lipoprotein lipase deficiency, however, long term data and larger patient numbers are required to establish their role regards CVD outcomes<sup>[105]</sup>.

Further elucidation, of the role of TG and CVD, may also occur with the introduction of non-HDL in the recent NICE guidance on lipid guidance and CVD risk assessment<sup>[3]</sup>. This will lead to postprandial hypertriglyceridaemia being represented in CVD risk assessment<sup>[3]</sup>. Data suggest that postprandial hypertriglyceridaemia is an independent CVD risk factor and that it is non-fasting TG, rather than fasting TG, that provide the main risk<sup>[106]</sup>. There is evidence that fibrates are useful in ameliorating vascular damage caused by postprandial elevations in TG level by targeting TG metabolism, although the evidence is not conclusive at the current time with robust outcome data lacking<sup>[107-109]</sup>.

## CONCLUSION

Clearly the evidence for fibrate use in CVD remains controversial with subgroup and *post hoc* analysis suggesting that PPAR $\alpha$  agonism by fibrates in those with low HDL-C and elevated TG levels, the atherogenic dyslipidaemia, could provide additional benefit. However, the blanket use of statins, suggested by guidelines, may contribute to the underlying dyslipidaemia and metabolic derangement being ignored and additional therapies not being offered. Statins should be the front-line agent in our view, except in patients with significantly elevated TG. However, there exists data that suggests that in patients with the atherogenic

dyslipidaemia, the dyslipidaemia seen in the metabolic syndrome, fibrates may have a role. This evidence is perhaps submerged by the data collected from statin RCTs. In the United States the prevalence of metabolic syndrome is about 40%<sup>[110]</sup>, therefore, small benefits in this group may have a large impact on the global CVD burden.

Besides CVD, other macrovascular and microvascular complications may be ameliorated by fibrates and therefore a holistic and individualised treatment plan is encouraged. What are required are trials looking at fibrates and statins in relevant groups designed to detect CVD benefits. Work may be aided by the development of high potency PPAR $\alpha$  agonists, the assumption being that greater efficacy at lipid reduction and increased cholesterol efflux may translate to greater and therefore universally detectable CVD benefit which would put the current controversy to rest<sup>[111]</sup>.

## REFERENCES

- 1 **Mathers CD**, Boerma T, Ma Fat D. Global and regional causes of death. *Br Med Bull* 2009; **92**: 7-32 [PMID: 19776034 DOI: 10.1093/bmb/ldp028]
- 2 **British Heart Foundation**. Cardiovascular disease statistics 2014. Oxford, United Kingdom: British Heart Foundation Centre on Population Approaches for Non-Communicable Disease Prevention, Nuffield Department of Population Health, University of Oxford, 2014
- 3 **National Institute for Health and Care Excellence**. Lipid modification: cardiovascular risk assessment and the modification of blood lipids for the primary and secondary prevention of cardiovascular disease. NICE guidelines [CG181] 2014. [Accessed 2015 Jun 14]. Available from: URL: <http://www.nice.org.uk/guidance/cg181>
- 4 **Baigent C**, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, Kirby A, Sourjina T, Peto R, Collins R, Simes R. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet* 2005; **366**: 1267-1278 [PMID: 16214597 DOI: 10.1016/S0140-6736(05)67394-1]
- 5 **Ridker PM**, Genest J, Boekholdt SM, Libby P, Gotto AM, Nordestgaard BG, Mora S, MacFadyen JG, Glynn RJ, Kastelein JJ. HDL cholesterol and residual risk of first cardiovascular events after treatment with potent statin therapy: an analysis from the JUPITER trial. *Lancet* 2010; **376**: 333-339 [PMID: 20655105 DOI: 10.1016/S0140-6736(10)60713-1]
- 6 **Pyörälä K**, Ballantyne CM, Gumbiner B, Lee MW, Shah A, Davies MJ, Mitchel YB, Pedersen TR, Kjekshus J. Reduction of cardiovascular events by simvastatin in nondiabetic coronary heart disease patients with and without the metabolic syndrome: subgroup analyses of the Scandinavian Simvastatin Survival Study (4S). *Diabetes Care* 2004; **27**: 1735-1740 [PMID: 15220255 DOI: 10.2337/diacare.27.7.1735]
- 7 **Eberly LE**, Stamler J, Neaton JD. Relation of triglyceride levels, fasting and nonfasting, to fatal and nonfatal coronary heart disease. *Arch Intern Med* 2003; **163**: 1077-1083 [PMID: 12742806 DOI: 10.1001/archinte.163.9.1077]
- 8 **Nordestgaard BG**, Benn M, Schnohr P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. *JAMA* 2007; **298**: 299-308 [PMID: 17635890 DOI: 10.1001/jama.298.3.299]
- 9 **Scharnagl H**, Schinker R, Gierens H, Nauck M, Wieland H, März W. Effect of atorvastatin, simvastatin, and lovastatin on the metabolism of cholesterol and triacylglycerides in HepG2 cells. *Biochem Pharmacol* 2001; **62**: 1545-1555 [PMID: 11728391 DOI:

- 10.1016/S0006-2952(01)00790-0]
- 10 Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet* 1994; **344**: 1383-1389 [PMID: 7968073]
  - 11 Reiner Z, Catapano AL, De Backer G, Graham I, Taskinen MR, Wiklund O, Agewall S, Alegria E, Chapman MJ, Durrington P, Erdine S, Halcox J, Hobbs R, Kjekshus J, Filardi PP, Riccardi G, Storey RF, Wood D. ESC/EAS Guidelines for the management of dyslipidaemias: the Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS). *Eur Heart J* 2011; **32**: 1769-1818 [PMID: 21712404 DOI: 10.1093/eurheartj/ehrl58]
  - 12 Waters DD, Guyton JR, Herrington DM, McGowan MP, Wenger NK, Shear C. Treating to New Targets (TNT) Study: does lowering low-density lipoprotein cholesterol levels below currently recommended guidelines yield incremental clinical benefit? *Am J Cardiol* 2004; **93**: 154-158 [PMID: 14715339]
  - 13 Nissen SE, Tuzcu EM, Schoenhagen P, Brown BG, Ganz P, Vogel RA, Crowe T, Howard G, Cooper CJ, Brodie B, Grines CL, DeMaria AN. Effect of intensive compared with moderate lipid-lowering therapy on progression of coronary atherosclerosis: a randomized controlled trial. *JAMA* 2004; **291**: 1071-1080 [PMID: 14996776 DOI: 10.1001/jama.291.9.1071]
  - 14 Cannon CP, McCabe CH, Belder R, Breen J, Braunwald E. Design of the Pravastatin or Atorvastatin Evaluation and Infection Therapy (PROVE IT)-TIMI 22 trial. *Am J Cardiol* 2002; **89**: 860-861 [PMID: 11909576 DOI: 10.1016/S0002-9149(02)02201-4]
  - 15 Nissen SE, Nicholls SJ, Sipahi I, Libby P, Raichlen JS, Ballantyne CM, Davignon J, Erbel R, Fruchart JC, Tardif JC, Schoenhagen P, Crowe T, Cain V, Wolski K, Goormastic M, Tuzcu EM. Effect of very high-intensity statin therapy on regression of coronary atherosclerosis: the ASTEROID trial. *JAMA* 2006; **295**: 1556-1565 [PMID: 16533939 DOI: 10.1001/jama.295.13.jpc60002]
  - 16 Nicholls SJ, Borgman M, Nissen SE, Raichlen JS, Ballantyne C, Barter P, Chapman MJ, Erbel R, Libby P. Impact of statins on progression of atherosclerosis: rationale and design of SATURN (Study of Coronary Atheroma by Intravascular Ultrasound: effect of Rosuvastatin versus Atorvastatin). *Curr Med Res Opin* 2011; **27**: 1119-1129 [PMID: 21446892 DOI: 10.1185/03007995.2011.570746]
  - 17 Barr DP, Russ EM, Eder HA. Protein-lipid relationships in human plasma. II. In atherosclerosis and related conditions. *Am J Med* 1951; **11**: 480-493 [PMID: 14885223 DOI: 10.1016/0002-9343(51)90183-0]
  - 18 Andersson C, Lyass A, Vasan RS, Massaro JM, D'Agostino RB, Robins SJ. Long-term risk of cardiovascular events across a spectrum of adverse major plasma lipid combinations in the Framingham Heart Study. *Am Heart J* 2014; **168**: 878-883.e1 [PMID: 25458651 DOI: 10.1016/j.ahj.2014.08.007]
  - 19 Assmann G, Cullen P, Schulte H. Simple scoring scheme for calculating the risk of acute coronary events based on the 10-year follow-up of the prospective cardiovascular Münster (PROCAM) study. *Circulation* 2002; **105**: 310-315 [PMID: 11804985 DOI: 10.1161/hc0302.102575]
  - 20 Assmann G, Cullen P, Schulte H. The Münster Heart Study (PROCAM). Results of follow-up at 8 years. *Eur Heart J* 1998; **19** Suppl A: A2-11 [PMID: 9519336]
  - 21 Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes* 1988; **37**: 1595-1607 [PMID: 3056758 DOI: 10.2337/diab.37.12.1595]
  - 22 ICD. 2015 ICD-10-CM Diagnosis Code. [Accessed 2015 Jul 14]. Available from: URL: <http://www.icd10data.com/ICD10CM/Codes/E00-E89/E70-E88/E88/E88.81>
  - 23 Strange RC, Shipman KE, Ramachandran S. Metabolic syndrome: A review of the role of vitamin D in mediating susceptibility and outcome. *World J Diabetes* 2015; **6**: 896-911 [PMID: 26185598 DOI: 10.4239/wjd.v6.i7.896]
  - 24 Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 1998; **15**: 539-553 [PMID: 9686693]
  - 25 Balkau B, Charles MA. Comment on the provisional report from the WHO consultation. European Group for the Study of Insulin Resistance (EGIR) *Diabet Med* 1999; **16**: 442-443 [PMID: 10342346 DOI: 10.1046/j.1464-5491.1999.00059.x]
  - 26 Einhorn D, Reaven GM, Cobin RH, Ford E, Ganda OP, Handelsman Y, Hellman R, Jellinger PS, Kendall D, Krauss RM, Neufeld ND, Petak SM, Rodbard HW, Seibel JA, Smith DA, Wilson PW. American College of Endocrinology position statement on the insulin resistance syndrome. *Endocr Pract* 2003; **9**: 237-252 [PMID: 12924350]
  - 27 National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* 2002; **106**: 3143-3421 [PMID: 12485966]
  - 28 Zimmet P, M M Alberti KG, Serrano Rios M. [A new international diabetes federation worldwide definition of the metabolic syndrome: the rationale and the results]. *Rev Esp Cardiol* 2005; **58**: 1371-1376 [PMID: 16371194 DOI: 10.1016/S1885-5857(06)60742-1]
  - 29 Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart JC, James WP, Loria CM, Smith SC. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation* 2009; **120**: 1640-1645 [PMID: 19805654 DOI: 10.1161/CIRCULATIONAHA.109.192644]
  - 30 Gami AS, Witt BJ, Howard DE, Erwin PJ, Gami LA, Somers VK, Montori VM. Metabolic syndrome and risk of incident cardiovascular events and death: a systematic review and meta-analysis of longitudinal studies. *J Am Coll Cardiol* 2007; **49**: 403-414 [PMID: 17258085 DOI: 10.1016/j.jacc.2006.09.032]
  - 31 Eddy DM, Schlessinger L, Heikes K. The metabolic syndrome and cardiovascular risk: implications for clinical practice. *Int J Obes (Lond)* 2008; **32** Suppl 2: S5-10 [PMID: 18469838 DOI: 10.1038/ijo.2008.28]
  - 32 Bayturan O, Tuzcu EM, Lavoie A, Hu T, Wolski K, Schoenhagen P, Kapadia S, Nissen SE, Nicholls SJ. The metabolic syndrome, its component risk factors, and progression of coronary atherosclerosis. *Arch Intern Med* 2010; **170**: 478-484 [PMID: 20212186 DOI: 10.1001/archinternmed.2009.551]
  - 33 Eckel RH, Alberti KG, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet* 2010; **375**: 181-183 [PMID: 20109902 DOI: 10.1016/S0140-6736(09)61794-3]
  - 34 Mathivat A, Cottet J. [Clinical trials on the hypocholesteremia-producing effect of 2-phenylbutyric acid]. *Bull Mem Soc Med Hop Paris* 1953; **69**: 1030-1048 [PMID: 13141149]
  - 35 Cottet J, Redel J, Krumm-heller C, Tricaud ME. [Hypocholesterolemic property of sodium phenylethylacetate (22 TH) in the rat]. *Bull Acad Natl Med* 1953; **137**: 441-442 [PMID: 13094180]
  - 36 Thorp JM, Waring WS. Modification of metabolism and distribution of lipids by ethyl chlorophenoxyisobutyrate. *Nature* 1962; **194**: 948-949 [PMID: 13921051 DOI: 10.1038/194948a0]
  - 37 Matzkies F, Schulzky D, Berg G. [Procetofen, a new lipid- and urine-acid-reducing substance]. *Fortschr Med* 1978; **96**: 1939-1941 [PMID: 700582]
  - 38 Olsson AG, Rössner S, Walldius G, Carlson LA. Effect of gemfibrozil on lipoprotein concentrations in different types of hyperlipoproteinaemia. *Proc R Soc Med* 1976; **69** Suppl 2: 28-31 [PMID: 190607]
  - 39 Olsson AG, Lang PD. Dose-response study of bezafibrate on serum lipoprotein concentrations in hyperlipoproteinemia. *Atherosclerosis* 1978; **31**: 421-428 [PMID: 215174 DOI: 10.1016/0021-9150(78)90137-5]
  - 40 Olsson AG, Orö L. Dose-response study of the effect of cipro-



- fibrate on serum lipoprotein concentrations in hyperlipoproteinaemia. *Atherosclerosis* 1982; **42**: 229-243 [PMID: 6951582 DOI: 10.1016/0021-9150(82)90153-8]
- 41 **Balendiran GK**, Rath N, Kotheimer A, Miller C, Zeller M, Rath NP. Biomolecular chemistry of isopropyl fibrates. *J Pharm Sci* 2012; **101**: 1555-1569 [PMID: 22246648 DOI: 10.1002/jps.23040]
  - 42 **Hess R**, Stäubli W, Riess W. Nature of the hepatomegalic effect produced by ethyl-chlorophenoxy-isobutyrate in the rat. *Nature* 1965; **208**: 856-858 [PMID: 5870099 DOI: 10.1038/208856a0]
  - 43 **Reddy JK**, Azarnoff DL, Hignite CE. Hypolipidaemic hepatic peroxisome proliferators form a novel class of chemical carcinogens. *Nature* 1980; **283**: 397-398 [PMID: 6766207 DOI: 10.1038/283397a0]
  - 44 **Blümcke S**, Schwartzkopff W, Lobeck H, Edmondson NA, Prentice DE, Blane GF. Influence of fenofibrate on cellular and subcellular liver structure in hyperlipidemic patients. *Atherosclerosis* 1983; **46**: 105-116 [PMID: 6838687 DOI: 10.1016/0021-9150(83)90169-7]
  - 45 WHO cooperative trial on primary prevention of ischaemic heart disease with clofibrate to lower serum cholesterol: final mortality follow-up. Report of the Committee of Principal Investigators. *Lancet* 1984; **2**: 600-604 [PMID: 6147641 DOI: 10.1016/S0140-6736(84)90595-6]
  - 46 **Shek A**, Ferrill MJ. Statin-fibrate combination therapy. *Ann Pharmacother* 2001; **35**: 908-917 [PMID: 11485144 DOI: 10.1345/aph.10315]
  - 47 **Davidson MH**, Armani A, McKenney JM, Jacobson TA. Safety considerations with fibrate therapy. *Am J Cardiol* 2007; **99**: 3C-18C [PMID: 17368275 DOI: 10.1016/j.amjcard.2006.11.016]
  - 48 **Reddy JK**, Goel SK, Nemali MR, Carrino JJ, Laffler TG, Reddy MK, Sperbeck SJ, Osumi T, Hashimoto T, Lalwani ND. Transcription regulation of peroxisomal fatty acyl-CoA oxidase and enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase in rat liver by peroxisome proliferators. *Proc Natl Acad Sci USA* 1986; **83**: 1747-1751 [PMID: 3456610 DOI: 10.1073/pnas.83.6.1747]
  - 49 **Staels B**, van Tol A, Verhoeven G, Auwerx J. Apolipoprotein A-IV messenger ribonucleic acid abundance is regulated in a tissue-specific manner. *Endocrinology* 1990; **126**: 2153-2163 [PMID: 2318160 DOI: 10.1210/endo-126-4-2153]
  - 50 **Issemann I**, Green S. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature* 1990; **347**: 645-650 [PMID: 2129546 DOI: 10.1038/347645a0]
  - 51 **Dreyer C**, Krey G, Keller H, Givel F, Helftenbein G, Wahli W. Control of the peroxisomal beta-oxidation pathway by a novel family of nuclear hormone receptors. *Cell* 1992; **68**: 879-887 [PMID: 1312391 DOI: 10.1016/0092-8674(92)90031-7]
  - 52 **Escher P**, Wahli W. Peroxisome proliferator-activated receptors: insight into multiple cellular functions. *Mutat Res* 2000; **448**: 121-138 [PMID: 10725467 DOI: 10.1016/S0027-5107(99)00231-6]
  - 53 **Ziouzenkova O**, Perrey S, Asatryan L, Hwang J, MacNaul KL, Moller DE, Rader DJ, Sevanian A, Zechner R, Hoefler G, Plutzky J. Lipolysis of triglyceride-rich lipoproteins generates PPAR ligands: evidence for an antiinflammatory role for lipoprotein lipase. *Proc Natl Acad Sci USA* 2003; **100**: 2730-2735 [PMID: 12606719 DOI: 10.1073/pnas.0538015100]
  - 54 **Juge-Aubry C**, Pernin A, Favez T, Burger AG, Wahli W, Meier CA, Desvergne B. DNA binding properties of peroxisome proliferator-activated receptor subtypes on various natural peroxisome proliferator response elements. Importance of the 5'-flanking region. *J Biol Chem* 1997; **272**: 25252-25259 [PMID: 9312141 DOI: 10.1074/jbc.272.40.25252]
  - 55 **Kersten S**. Peroxisome proliferator activated receptors and lipoprotein metabolism. *PPAR Res* 2008; **2008**: 132960 [PMID: 18288277 DOI: 10.1155/2008/132960]
  - 56 **Duval C**, Müller M, Kersten S. PPARalpha and dyslipidemia. *Biochim Biophys Acta* 2007; **1771**: 961-971 [PMID: 17604218 DOI: 10.1016/j.bbali.2007.05.003]
  - 57 **Schoonjans K**, Peinado-Onsurbe J, Lefebvre AM, Heyman RA, Briggs M, Deeb S, Staels B, Auwerx J. PPARalpha and PPARgamma activators direct a distinct tissue-specific transcriptional response via a PPARE in the lipoprotein lipase gene. *EMBO J* 1996; **15**: 5336-5348 [PMID: 8895578]
  - 58 **Vu-Dac N**, Gervois P, Jakel H, Nowak M, Bauge E, Dehondt H, Staels B, Pennacchio LA, Rubin EM, Fruchart-Najib J, Fruchart JC. Apolipoprotein A5, a crucial determinant of plasma triglyceride levels, is highly responsive to peroxisome proliferator-activated receptor alpha activators. *J Biol Chem* 2003; **278**: 17982-17985 [PMID: 12637506 DOI: 10.1074/jbc.M212191200]
  - 59 **Farnier M**. Update on the clinical utility of fenofibrate in mixed dyslipidemias: mechanisms of action and rational prescribing. *Vasc Health Risk Manag* 2008; **4**: 991-1000 [PMID: 19183747 DOI: 10.2147/VHRM.S3390]
  - 60 **Ramirez C**. Hypertriglyceridemia and Acute Pancreatitis - How Fearful Should we be of Pushing the Limits? [Accessed 2015 Oct 28]. Available from: URL: <https://www.utexas.edu/pharmacy/divisions/pharmaco/rounds/ramirez09-13-2013.pdf>
  - 61 **Frick MH**, Elo O, Haapa K, Heinonen OP, Heinsalmi P, Helo P, Huttunen JK, Kaitaniemi P, Koskinen P, Manninen V. Helsinki Heart Study: primary-prevention trial with gemfibrozil in middle-aged men with dyslipidemia. Safety of treatment, changes in risk factors, and incidence of coronary heart disease. *N Engl J Med* 1987; **317**: 1237-1245 [PMID: 3313041 DOI: 10.1056/NEJM198711123172001]
  - 62 **Rubins HB**, Robins SJ, Collins D, Fye CL, Anderson JW, Elam MB, Faas FH, Linares E, Schaefer EJ, Schectman G, Wilt TJ, Wittes J. Gemfibrozil for the secondary prevention of coronary heart disease in men with low levels of high-density lipoprotein cholesterol. Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial Study Group. *N Engl J Med* 1999; **341**: 410-418 [PMID: 10438259 DOI: 10.1056/NEJM199908053410604]
  - 63 **Keech A**, Simes RJ, Barter P, Best J, Scott R, Taskinen MR, Forder P, Pillai A, Davis T, Glasziou P, Drury P, Kesäniemi YA, Sullivan D, Hunt D, Colman P, d'Emden M, Whiting M, Ehnholm C, Laakso M. Effects of long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD study): randomised controlled trial. *Lancet* 2005; **366**: 1849-1861 [PMID: 16310551 DOI: 10.1016/S0140-6736(05)67667-2]
  - 64 **Ginsberg HN**, Elam MB, Lovato LC, Crouse JR, Leiter LA, Linz P, Friedewald WT, Buse JB, Gerstein HC, Probstfield J, Grimm RH, Ismail-Beigi F, Bigger JT, Goff DC, Cushman WC, Simons-Morton DG, Byington RP. Effects of combination lipid therapy in type 2 diabetes mellitus. *N Engl J Med* 2010; **362**: 1563-1574 [PMID: 20228404 DOI: 10.1056/NEJMoa1001282]
  - 65 **Bezafibrate Infarction Prevention (BIP) study**. Secondary prevention by raising HDL cholesterol and reducing triglycerides in patients with coronary artery disease. *Circulation* 2000; **102**: 21-27 [PMID: 10880410 DOI: 10.1161/01.CIR.102.1.21]
  - 66 **Manninen V**, Tenkanen L, Koskinen P, Huttunen JK, Mänttari M, Heinonen OP, Frick MH. Joint effects of serum triglyceride and LDL cholesterol and HDL cholesterol concentrations on coronary heart disease risk in the Helsinki Heart Study. Implications for treatment. *Circulation* 1992; **85**: 37-45 [PMID: 1728471 DOI: 10.1161/01.CIR.85.1.37]
  - 67 **Tenkanen L**, Mänttari M, Manninen V. Some coronary risk factors related to the insulin resistance syndrome and treatment with gemfibrozil. Experience from the Helsinki Heart Study. *Circulation* 1995; **92**: 1779-1785 [PMID: 7671361 DOI: 10.1161/01.CIR.92.7.1779]
  - 68 **Heart Protection Study Collaborative Group**. MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20,536 high-risk individuals: a randomised placebo-controlled trial. *Lancet* 2002; **360**: 7-22 [PMID: 12114036 DOI: 10.1016/S0140-6736(02)09327-3]
  - 69 **Scott R**, O'Brien R, Fulcher G, Pardy C, D'Emden M, Tse D, Taskinen MR, Ehnholm C, Keech A. Effects of fenofibrate treatment on cardiovascular disease risk in 9,795 individuals with type 2 diabetes and various components of the metabolic syndrome: the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study. *Diabetes Care* 2009; **32**: 493-498 [PMID: 18984774 DOI: 10.2337/dc08-1543]
  - 70 Effect of fenofibrate on progression of coronary-artery disease in

- type 2 diabetes: the Diabetes Atherosclerosis Intervention Study, a randomised study. *Lancet* 2001; **357**: 905-910 [PMID: 11289345 DOI: 10.1016/S0140-6736(00)04209-4]
- 71 **Davidson MH**, Rosenson RS, Maki KC, Nicholls SJ, Ballantyne CM, Mazzone T, Carlson DM, Williams LA, Kelly MT, Camp HS, Lele A, Stolzenbach JC. Effects of fenofibric acid on carotid intima-media thickness in patients with mixed dyslipidemia on atorvastatin therapy: randomized, placebo-controlled study (FIRST). *Arterioscler Thromb Vasc Biol* 2014; **34**: 1298-1306 [PMID: 24743431 DOI: 10.1161/ATVBAHA.113.302926]
  - 72 **Tenenbaum A**, Motro M, Fisman EZ, Tanne D, Boyko V, Behar S. Bezafibrate for the secondary prevention of myocardial infarction in patients with metabolic syndrome. *Arch Intern Med* 2005; **165**: 1154-1160 [PMID: 15911729 DOI: 10.1001/archinte.165.10.1154]
  - 73 **Ericsson CG**, Hamsten A, Nilsson J, Grip L, Svane B, de Faire U. Angiographic assessment of effects of bezafibrate on progression of coronary artery disease in young male postinfarction patients. *Lancet* 1996; **347**: 849-853 [PMID: 8622389]
  - 74 **Meade T**, Zuhrie R, Cook C, Cooper J. Bezafibrate in men with lower extremity arterial disease: randomised controlled trial. *BMJ* 2002; **325**: 1139 [PMID: 12433762 DOI: 10.1136/bmj.325.7373.1139]
  - 75 **Wang R**, Lagakos SW, Ware JH, Hunter DJ, Drazen JM. Statistics in medicine--reporting of subgroup analyses in clinical trials. *N Engl J Med* 2007; **357**: 2189-2194 [PMID: 18032770 DOI: 10.1056/NEJMs077003]
  - 76 **Rubins HB**, Robins SJ, Collins D, Nelson DB, Elam MB, Schaefer EJ, Faas FH, Anderson JW. Diabetes, plasma insulin, and cardiovascular disease: subgroup analysis from the Department of Veterans Affairs high-density lipoprotein intervention trial (VA-HIT). *Arch Intern Med* 2002; **162**: 2597-2604 [PMID: 12456232 DOI: 10.1001/archinte.162.22.2597]
  - 77 **Bruckert E**, Labreuche J, Deplanque D, Touboul PJ, Amarenco P. Fibrates effect on cardiovascular risk is greater in patients with high triglyceride levels or atherogenic dyslipidemia profile: a systematic review and meta-analysis. *J Cardiovasc Pharmacol* 2011; **57**: 267-272 [PMID: 21052016 DOI: 10.1097/FJC.0b013e318202709f]
  - 78 **Jun M**, Foote C, Lv J, Neal B, Patel A, Nicholls SJ, Grobbee DE, Cass A, Chalmers J, Perkovic V. Effects of fibrates on cardiovascular outcomes: a systematic review and meta-analysis. *Lancet* 2010; **375**: 1875-1884 [PMID: 20462635 DOI: 10.1016/S0140-6736(10)60656-3]
  - 79 **d'Emden MC**, Jenkins AJ, Li L, Zannino D, Mann KP, Best JD, Stuckey BG, Park K, Saltevo J, Keech AC. Favourable effects of fenofibrate on lipids and cardiovascular disease in women with type 2 diabetes: results from the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study. *Diabetologia* 2014; **57**: 2296-2303 [PMID: 25149070 DOI: 10.1007/s00125-014-3344-3]
  - 80 **Frick MH**, Heinonen OP, Huttunen JK, Koskinen P, Mänttari M, Manninen V. Efficacy of gemfibrozil in dyslipidaemic subjects with suspected heart disease. An ancillary study in the Helsinki Heart Study frame population. *Ann Med* 1993; **25**: 41-45 [PMID: 8435186]
  - 81 **Burgess DC**, Hunt D, Li L, Zannino D, Williamson E, Davis TM, Laakso M, Kesäniemi YA, Zhang J, Sy RW, Lehto S, Mann S, Keech AC. Incidence and predictors of silent myocardial infarction in type 2 diabetes and the effect of fenofibrate: an analysis from the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study. *Eur Heart J* 2010; **31**: 92-99 [PMID: 19797259 DOI: 10.1093/eurheartj/ehp377]
  - 82 **Kühnast S**, Fiocco M, van der Hooft JW, Princen HM, Jukema JW. Innovative pharmaceutical interventions in cardiovascular disease: Focusing on the contribution of non-HDL-C/LDL-C-lowering versus HDL-C-raising: A systematic review and meta-analysis of relevant preclinical studies and clinical trials. *Eur J Pharmacol* 2015; **763**: 48-63 [PMID: 25989133 DOI: 10.1016/j.ejphar.2015.03.089]
  - 83 **Davis TM**, Ting R, Best JD, Donoghoe MW, Drury PL, Sullivan DR, Jenkins AJ, O'Connell RL, Whiting MJ, Glasziou PP, Simes RJ, Kesäniemi YA, GebSKI VJ, Scott RS, Keech AC. Effects of fenofibrate on renal function in patients with type 2 diabetes mellitus: the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) Study. *Diabetologia* 2011; **54**: 280-290 [PMID: 21052978 DOI: 10.1007/s00125-010-1951-1]
  - 84 **Keech AC**, Mitchell P, Summanen PA, O'Day J, Davis TM, Moffitt MS, Taskinen MR, Simes RJ, Tse D, Williamson E, Merrifield A, Laatikainen LT, d'Emden MC, Crimet DC, O'Connell RL, Colman PG. Effect of fenofibrate on the need for laser treatment for diabetic retinopathy (FIELD study): a randomised controlled trial. *Lancet* 2007; **370**: 1687-1697 [PMID: 17988728 DOI: 10.1016/S0140-6736(07)61607-9]
  - 85 **Chew EY**, Ambrosius WT, Davis MD, Danis RP, Gangaputra S, Greven CM, Hubbard L, Esser BA, Lovato JF, Perdue LH, Goff DC, Cushman WC, Ginsberg HN, Elam MB, Genuth S, Gerstein HC, Schubart U, Fine LJ. Effects of medical therapies on retinopathy progression in type 2 diabetes. *N Engl J Med* 2010; **363**: 233-244 [PMID: 20587587 DOI: 10.1056/NEJMoa1001288]
  - 86 **Ramachandran S**, Abbas A, Saraf S, Raju J, Jewkes C, Jones AF. Significant increase in high-density lipoprotein cholesterol with fibrates is associated with low pretreatment high-density lipoprotein cholesterol: findings from an outpatient clinic setting. *Metab Syndr Relat Disord* 2012; **10**: 189-194 [PMID: 22283634 DOI: 10.1089/met.2011.0112]
  - 87 **Collins MW**, König CS, Abbas A, Jewkes C, Jones AF, Ramachandran S. Association between triglyceride and high-density lipoprotein cholesterol change following fibrate therapy. *Diabetes Metab Syndr* 2014; **8**: 212-215 [PMID: 25301006 DOI: 10.1016/j.dsx.2014.09.004]
  - 88 **Kornitzer M**, Dramaix M, Vandenbroek MD, Everaert L, Gerlinger C. Efficacy and tolerance of 200 mg micronised fenofibrate administered over a 6-month period in hyperlipidaemic patients: an open Belgian multicenter study. *Atherosclerosis* 1994; **110** Suppl: S49-S54 [PMID: 7857385 DOI: 10.1016/0021-9150(94)05378-V]
  - 89 **Scholz M**, Tselmin S, Fischer S, Julius U. Hypertriglyceridemia in an outpatient department--Significance as an atherosclerotic risk factor. *Atheroscler Suppl* 2015; **18**: 146-153 [PMID: 25936319 DOI: 10.1016/j.atherosclerosis.2015.02.011]
  - 90 **Rohatgi A**, Khera A, Berry JD, Givens EG, Ayers CR, Wedin KE, Neeland IJ, Yuhanna IS, Rader DR, de Lemos JA, Shaul PW. HDL cholesterol efflux capacity and incident cardiovascular events. *N Engl J Med* 2014; **371**: 2383-2393 [PMID: 25404125 DOI: 10.1056/NEJMoa1409065]
  - 91 **Chandra A**, Neeland IJ, Das SR, Khera A, Turer AT, Ayers CR, McGuire DK, Rohatgi A. Relation of black race between high density lipoprotein cholesterol content, high density lipoprotein particles and coronary events (from the Dallas Heart Study). *Am J Cardiol* 2015; **115**: 890-894 [PMID: 25661572 DOI: 10.1016/j.amjcard.2015.01.015]
  - 92 **Gandhi N**, Lenton R, Bhartia M, Abbas A, Raju J, Ramachandran S. Effect of fibrate treatment on liver function tests in patients with the metabolic syndrome. *Springerplus* 2014; **3**: 14 [PMID: 24455467 DOI: 10.1186/2193-1801-3-14]
  - 93 **Day CP**, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology* 1998; **114**: 842-845 [PMID: 9547102 DOI: 10.1016/S0016-5085(98)70599-2]
  - 94 **Day CP**. From fat to inflammation. *Gastroenterology* 2006; **130**: 207-210 [PMID: 16401483 DOI: 10.1053/j.gastro.2005.11.017]
  - 95 **Jou J**, Choi SS, Diehl AM. Mechanisms of disease progression in nonalcoholic fatty liver disease. *Semin Liver Dis* 2008; **28**: 370-379 [PMID: 18956293 DOI: 10.1055/s-0028-1091981]
  - 96 **Downman JK**, Tomlinson JW, Newsome PN. Pathogenesis of non-alcoholic fatty liver disease. *QJM* 2010; **103**: 71-83 [PMID: 19914930 DOI: 10.1093/qjmed/hcp158]
  - 97 **Abbas A**, Saraf S, Ramachandran S, Raju J, Ramachandran S. Fibrates and estimated glomerular filtration rate: observations from an outpatient clinic setting and clinical implications. *Postgrad Med J* 2012; **88**: 503-506 [PMID: 22460858 DOI: 10.1136/postgradmedj-2011-130594]
  - 98 **Abbas A**, Saraf S, Ramachandran S, Raju J, Ramachandran S. Factors associated with fibrate-induced creatinine elevation:

- Observations in an outpatient setting. *World J Nephrol Urol* 2012; **1**: 51-58 [DOI: 10.4021/wjnu27w]
- 99 **Chandler HA**, Batchelor AJ. Ciprofibrate and lipid profile. *Lancet* 1994; **344**: 128-129 [PMID: 7912363 DOI: 10.1016/S0140-6736(94)91314-5]
  - 100 **Ebcioglu Z**, Morgan J, Carey C, Capuzzi D. Paradoxical lowering of high-density lipoprotein cholesterol level in 2 patients receiving fenofibrate and a thiazolidinedione. *Ann Intern Med* 2003; **139**: W80 [PMID: 14597479]
  - 101 **Shetty C**, Balasubramani M, Capps N, Milles J, Ramachandran S. Paradoxical HDL-C reduction during rosiglitazone and fibrate treatment. *Diabet Med* 2007; **24**: 94-97 [PMID: 17227331 DOI: 10.1111/j.1464-5491.2007.02029.x]
  - 102 **Saraf S**, Nishtala S, Parretti H, Capps N, Ramachandran S. Paradoxical fall in HDL cholesterol observed in a patient treated with rosiglitazone and pioglitazone. *Br J of Diabetes and Vascular Medicine* 2009; **9**: 186-189 [DOI: 10.1177/1474651409341325]
  - 103 **Keidar S**, Guttman H, Stam T, Fishman I, Shapira C. High incidence of reduced plasma HDL cholesterol in diabetic patients treated with rosiglitazone and fibrate. *Pharmacoevidiol Drug Saf* 2007; **16**: 1192-1194 [PMID: 17636555 DOI: 10.1002/pds.1448]
  - 104 **Zhang LH**, Kamanna VS, Ganji SH, Xiong XM, Kashyap ML. Pioglitazone increases apolipoprotein A-I production by directly enhancing PPRE-dependent transcription in HepG2 cells. *J Lipid Res* 2010; **51**: 2211-2222 [PMID: 20371549 DOI: 10.1194/jlr.M004481]
  - 105 **Scott LJ**. Alipogene tiparvovec: a review of its use in adults with familial lipoprotein lipase deficiency. *Drugs* 2015; **75**: 175-182 [PMID: 25559420 DOI: 10.1007/s40265-014-0339-9]
  - 106 **Bansal S**, Buring JE, Rifai N, Mora S, Sacks FM, Ridker PM. Fasting compared with nonfasting triglycerides and risk of cardiovascular events in women. *JAMA* 2007; **298**: 309-316 [PMID: 17635891 DOI: 10.1001/jama.298.3.309]
  - 107 **Ohno Y**, Miyoshi T, Noda Y, Oe H, Toh N, Nakamura K, Kohno K, Morita H, Ito H. Bezafibrate improves postprandial hypertriglyceridemia and associated endothelial dysfunction in patients with metabolic syndrome: a randomized crossover study. *Cardiovasc Diabetol* 2014; **13**: 71 [PMID: 24708775 DOI: 10.1186/1475-2840-13-71]
  - 108 **Hogue JC**, Lamarche B, Deshaies Y, Tremblay AJ, Bergeron J, Gagné C, Couture P. Differential effect of fenofibrate and atorvastatin on in vivo kinetics of apolipoproteins B-100 and B-48 in subjects with type 2 diabetes mellitus with marked hypertriglyceridemia. *Metabolism* 2008; **57**: 246-254 [PMID: 18191056 DOI: 10.1016/j.metabol.2007.09.008]
  - 109 **Watts GF**, Barrett PH, Ji J, Serone AP, Chan DC, Croft KD, Loehrer F, Johnson AG. Differential regulation of lipoprotein kinetics by atorvastatin and fenofibrate in subjects with the metabolic syndrome. *Diabetes* 2003; **52**: 803-811 [PMID: 12606523]
  - 110 **Ford ES**. Prevalence of the metabolic syndrome defined by the International Diabetes Federation among adults in the U.S. *Diabetes Care* 2005; **28**: 2745-2749 [PMID: 16249550 DOI: 10.2337/diacare.28.11.2745]
  - 111 **Fruchart JC**. Selective peroxisome proliferator-activated receptor  $\alpha$  modulators (SPPARM $\alpha$ ): the next generation of peroxisome proliferator-activated receptor  $\alpha$ -agonists. *Cardiovasc Diabetol* 2013; **12**: 82 [PMID: 23721199 DOI: 10.1186/1475-2840-12-82]
  - 112 **Zimmet P**, Alberti KG, Kaufman F, Tajima N, Silink M, Arslanian S, Wong G, Bennett P, Shaw J, Caprio S. The metabolic syndrome in children and adolescents - an IDF consensus report. *Pediatr Diabetes* 2007; **8**: 299-306 [PMID: 17850473 DOI: 10.1111/j.1399-5448.2007.00271.x]
  - 113 **Grundy SM**, Brewer HB, Cleeman JI, Smith SC, Lenfant C. Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Arterioscler Thromb Vasc Biol* 2004; **24**: e13-e18 [PMID: 14766739 DOI: 10.1161/01.ATV.0000111245.75752.C6]

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## Role of vitamin D in diabetes mellitus and chronic kidney disease

Akio Nakashima, Keitaro Yokoyama, Takashi Yokoo, Mitsuyoshi Urashima

Akio Nakashima, Keitaro Yokoyama, Takashi Yokoo, Division of Nephrology and Hypertension, Department of Internal Medicine, Jikei University School of Medicine, Tokyo 105-8461, Japan

Akio Nakashima, Mitsuyoshi Urashima, Division of Molecular Epidemiology, Jikei University School of Medicine, Tokyo 105-8461, Japan

**Author contributions:** Nakashima A, Yokoyama K and Yokoo T contributed equally to the work performing the literature review and writing the manuscript; Urashima M provided support with proofing and editing paper; all authors reviewed and edited the manuscript.

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**Correspondence to:** Mitsuyoshi Urashima, MD, PhD, MPH, Division of Molecular Epidemiology, Jikei University School of Medicine, 3-25-8 Nishi-shimbashi, Minato-ku, Tokyo 105-8461, Japan. [urashima@jikei.ac.jp](mailto:urashima@jikei.ac.jp)  
Telephone: +81-3-34331111  
Fax: +81-3-54001250

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### Abstract

Approximately 30%-50% of people are recognized to have low levels of vitamin D, and insufficiency and deficiency of vitamin D are recognized as global health problems worldwide. Although the presence of hypovitamin D increases the risk of rickets and fractures, low vitamin D levels are also associated with hypertension, cancer, and cardiovascular disease. In addition, diabetes mellitus (DM) and chronic kidney disease (CKD) are also related to vitamin D levels. Vitamin D deficiency has been linked to onset and progression of DM. Although in patients with DM the relationship between vitamin D and insulin secretion, insulin resistance, and  $\beta$ -cell dysfunction are pointed out, evidence regarding vitamin D levels and DM is contradictory, and well controlled studies are needed. In addition, vitamin D influences the renin-angiotensin system, inflammation, and mineral bone disease, which may be associated with the cause and progression CKD. There is increasing evidence that vitamin D deficiency may be a risk factor for DM and CKD; however, it remains uncertain whether vitamin D deficiency also predisposes to death from DM and CKD. Although at this time, supplementation with vitamin D has not been shown to improve glycemic control or prevent incident DM, clinical trials with sufficient sample size, study periods, and optimal doses of vitamin D supplementation are still needed. This review focuses on the mechanism of vitamin D insufficiency and deficiency in DM or CKD, and discusses the current evidence regarding supplementation with vitamin D in patients with these diseases.

**Key words:** Vitamin D; Vitamin D deficiency; Diabetes mellitus; Chronic kidney disease; Cardiovascular disease

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**Core tip:** Vitamin D plays an essential role in diabetes



mellitus (DM) and chronic kidney disease (CKD). The relationship between vitamin D and insulin secretion, insulin resistance, and  $\beta$ -cell dysfunction are pointed out. Vitamin D deficiency has been linked with the renin-angiotensin system and inflammation, which may be associated with the cause and progression CKD. There is increasing evidence that vitamin D deficiency may be a risk factor for DM and CKD. Clinical trials with sufficient sample size, study periods, and optimal doses of vitamin D supplementation are still needed.

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## INTRODUCTION

Diabetes mellitus (DM) and chronic kidney disease (CKD) are common diseases worldwide, and their prevalence continues to increase<sup>[1,2]</sup>. Vitamin D deficiency is also recognized as a worldwide health problem<sup>[3]</sup>, and is associated with rickets and fracture. In addition, hypovitamin D has recently been considered a responsible factor in the onset and progression of DM and CKD. There has been increasing evidence suggesting that an inverse vitamin D status is prevalent in patients with DM or CKD<sup>[4]</sup>. Furthermore, supplementation of vitamin D in patients with DM or CKD has been reported in several trials and a meta-analysis<sup>[5]</sup>. In this review, we provide current clinical data on the mechanism of vitamin D deficiency and the effects of vitamin D on patients with DM or CKD.

## VITAMIN D PHYSIOLOGY

Vitamin D is a fat-soluble steroid hormone derived from dietary intake as well as synthesis through the skin *via* exposure to sunlight (Figure 1). Vitamin D<sub>3</sub> (cholecalciferol) and vitamin D<sub>2</sub> (ergocalciferol) are produced through solar ultraviolet B radiation (UVB; wavelength 290 to 315 nm). Vitamin D<sub>3</sub> is manufactured from previtamin D<sub>3</sub>, which is changed through UVB irradiation from provitamin D<sub>3</sub><sup>[6]</sup>. Most 25-hydroxyvitamin (25[OH]D) is derived from skin conversion. An alternative source is from dietary intake, mainly from foods of plant or animal origin. In general, animals and fish contain vitamin D<sub>3</sub>, and mushrooms contain vitamin D<sub>2</sub><sup>[7]</sup>. Vitamin D from the skin and diet is either stored in adipose tissue or converted to 25(OH)D in the liver. Vitamin D metabolism requires two hydroxylations to form its active metabolite. The first hydroxylation of vitamin D takes place in the liver where vitamin D is metabolized to 25(OH)D by cytochrome P 2R1 (CYP2R1). 25(OH)D binds to vitamin D-binding protein (DBP) and can flow into the blood in a stable form. 25(OH)D-

DBP complex is excreted into the urine and reabsorbed through megalin, a multiligand scavenger receptor in the proximal tubules<sup>[8,9]</sup>, where the complex is converted by 25-hydroxyvitamin D-1 $\alpha$ -hydroxylase (CYP27B1) and changed to its active form 1,25-dihydroxyvitamin (OH)<sub>2</sub>D, although other tissues have 1 $\alpha$ -hydroxylase enzymatic activity<sup>[10]</sup>. CYP27B1 gene expression in the kidney is mediated by various factors. Parathyroid hormone (PTH), hypocalcemia, hypophosphatemia, and calcitonin affect the activation of CYP27B1 and can increase 1,25-(OH)<sub>2</sub>D levels. On the other hand, 1,25-(OH)<sub>2</sub>D and fibroblast growth factor-23 (FGF-23) inhibit CYP27B1 and can decrease 1,25-(OH)<sub>2</sub>D levels<sup>[11]</sup>.

The binding of 1,25(OH)<sub>2</sub>D to the vitamin D receptor (VDR) in the nuclear receptor affects gene transcription. In general, 1,25(OH)<sub>2</sub>D promotes dietary calcium and phosphorus absorption in the intestine and regulates reabsorption of calcium in the renal tubules. Because VDR is expressed in a variety of organs, such as the heart, liver, blood vessels, and the central nervous system, 25-hydroxyvitamin D-1 $\alpha$ -hydroxylase is also expressed in these tissues<sup>[12]</sup>.

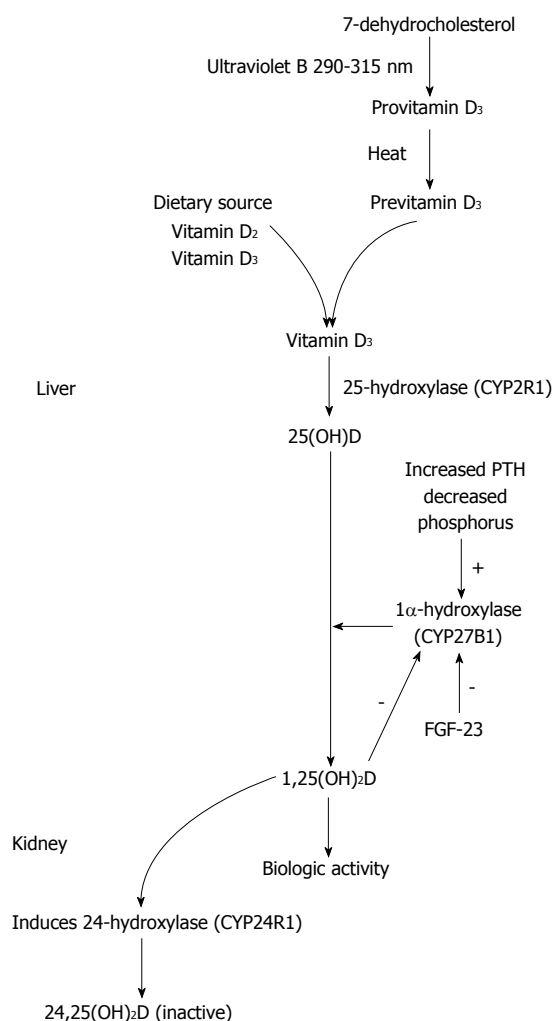
It is widely believed that 25(OH)D is the only precursor of 1,25(OH)<sub>2</sub>D and does not influence individual tissues. However, recent reports revealed that 25(OH)D has a weak binding capacity for VDR and affects several tissues in the autocrine or paracrine system<sup>[13,14]</sup>. In addition, extrarenal 1 $\alpha$ -hydroxylase enzymatic activity is controlled in different ways that that in renal tubular cells<sup>[15]</sup>.

## EPIDEMIOLOGY OF VITAMIN D

### DEFICIENCY

Because 1,25(OH)<sub>2</sub>D has a short half-life (approximately 15 h), 1,25(OH)<sub>2</sub>D levels are not considered a good indicator of vitamin D levels. As 25(OH)D is more stable in the blood than 1,25(OH)<sub>2</sub>D, blood concentrations of 25(OH)D are 500 to 1000 times higher than 1,25(OH)<sub>2</sub>D concentrations. Therefore, to evaluate vitamin D deficiency and insufficiency, serum 25(OH)D concentrations are considered an adequate biomarker. The United States Institute of Medicine defines vitamin D deficiency as 25(OH)D levels less than 20 ng/mL and greater than 20 ng/mL is sufficient upon evidence related to bone health<sup>[16]</sup>. Several studies reported that people with 25(OH)D levels less than 20 ng/mL is the risk factor of fracture<sup>[17]</sup> and have greater subsequent rates of bone loss<sup>[18]</sup>. On the other hand, the Endocrine Society's guidelines, which are based on patients with endocrine disorders, define vitamin D insufficiency as 25(OH)D levels of 21-29 ng/mL<sup>[19,20]</sup>. Despite these different definitions, both guidelines agree that vitamin D insufficiency and deficiency are common problems in certain populations.

About 1 billion people worldwide lack vitamin D<sup>[21,22]</sup>. Vitamin D deficiency and insufficiency are prevalent conditions not only in elderly people but also



**Figure 1 Mechanism of vitamin D synthesis.** FGF-23: Fibroblast growth factor-23.

in adolescents<sup>[23]</sup> and children<sup>[24]</sup>. One study reported that almost one half of participants had 25(OH)D levels less than 40 nmol/L during the winter and spring<sup>[25]</sup>. In this study, 7437 people from a British birth cohort study who were 45 years old had 25(OH)D levels measured. Although the prevalence of hypovitamin D, defined as levels below 40 nmol/L, was 15.4% during the spring and summer, the proportion was 46.6% during the winter and autumn. Other studies showed that vitamin D deficiency was especially common in older persons (67-95 years)<sup>[26,27]</sup>, and more than 50% of postmenopausal women taking medication for osteoporosis had 25(OH)D levels below 30 ng/mL<sup>[28]</sup>. Various factors, including age, sex, location, nutrition status, and physical fitness, affect vitamin D status<sup>[29]</sup>. In addition, diabetes, renal function, hypoalbuminemia, and albuminuria are also risk factors for vitamin D deficiency<sup>[30,31]</sup>.

Recently, the relationship between 25(OH)D levels and genetic polymorphisms of DBP were reported<sup>[32]</sup>. It was previously known that 25(OH)D concentrations differed between black Americans and whites<sup>[33]</sup>. Although it was generally thought that nutritional, environmental,

and hormonal factors affected racial differences<sup>[34]</sup>, the detailed mechanisms behind these differences are unknown. Powe *et al.*<sup>[32]</sup> reported that although total 25(OH)D and DBP were lower in black subjects than in white subjects, concentrations of estimated bioavailable 25(OH)D were similar between black and white subjects. In addition, because the affinity of DBP to 25(OH)D differs in the DBP gene polymorphism, genetic polymorphisms of DBP genes (rs7041 and rs4588) provide a likely explanation for racial variations in levels of DBP and 25(OH)D<sup>[35]</sup>. The combination of rs7041 and rs4588 produces amino acid changes resulting in variant DBPs (Gc1F, Gc1S, and Gc2). The phenotype of Gc1F, which is common in black homozygotes, was associated with the lowest levels of DBP (Gc1F/Gc1F homozygotes). On the other hand, Gc1S, which is common in white subjects, was associated with the highest DBP levels (Gc1S/Gc1S homozygotes). The Gc2/Gc2 homozygotes and Gc1F/Gc1S heterozygotes were associated with intermediate DBP levels. These findings suggest that racial differences in the distribution of DBP and total 25(OH)D are caused by DBP polymorphisms, and low total 25(OH)D levels do not indicate vitamin D deficiency. For purposes of cross-racial evaluations of vitamin D deficiency, it might be appropriate to estimate serum total 25(OH)D concentrations using DBP polymorphisms and DBP.

Associations between vitamin D levels and mortality have been shown by several observational studies<sup>[36,37]</sup>. Low vitamin D levels have also been shown to be associated with obesity, fractures, and infections<sup>[38]</sup>. Several observational studies have revealed potential links between low vitamin D levels and cardiovascular disease<sup>[39]</sup>. It is well known that people who live at high altitudes are at higher risk for hypertension and cardiovascular disease<sup>[40,41]</sup>. In a study of patients with hypertension who were exposed to UVB radiation three times a week for 3 mo, 25(OH)D concentrations increased by about 180%, and blood pressure became normal<sup>[42]</sup>. A prospective, nested, case-control study of 1484 women without hypertension and with low 25(OH)D levels showed that women with lower 25(OH)D levels had a higher rate of incident hypertension than controls. Low 25(OH)D concentrations have been shown to be inversely related to developing hypertension<sup>[43]</sup>. A recent Mendelian randomization study of vitamin D status and blood pressure concluded that increased plasma concentrations of 25(OH)D might reduce the risk for hypertension<sup>[44]</sup>. Cardiovascular disease such as coronary arterial disease<sup>[45]</sup>, myocardial infarction<sup>[46]</sup>, heart failure<sup>[47]</sup>, and stroke<sup>[48]</sup> are also associated with vitamin D deficiency. However, a recent study showed that high levels of 25(OH)D were also associated with cardiovascular disease mortality<sup>[49]</sup>. This prospective, observational, cohort study analyzed 247574 citizens from Denmark and showed that a 25(OH)D level below 12.5 nmol/L was associated with a higher risk for mortality [hazard ratio (HR) = 1.59] compared with the reference range (50-75 nmol/L); however, those with

levels higher than 125 nmol/L had the highest mortality risk (HR = 1.95). There is a possibility that maintaining adequate vitamin D levels is essential for human health.

As mentioned above, vitamin D status and cardiovascular disease are strongly associated. Animal models offer several mechanisms to explain this association. Activation of the renin-angiotensin-aldosterone system (RAAS) has been seen in VDR knockout mice<sup>[50]</sup>, and vitamin D has been shown to regulate the nuclear factor kappa beta pathway in renal failure model mice<sup>[51]</sup>. In vascular endothelial cells, transcription of nitric oxide synthase has been shown to be inhibited by vitamin D in mice<sup>[52]</sup>. In addition, vitamin D has been shown to activate the Keap1-Nrf pathway, which opposes oxidative stress, in renal failure model mice<sup>[53]</sup>.

## VITAMIN D AND DM

### Type 1 DM

Type 1 DM is caused by a complex autoimmune destruction of pancreatic islet  $\beta$ -cells, leading to absolute insulin deficiency. The autoimmune nature of type 1 DM has been clarified with the detection of auto-antibodies against islet  $\beta$ -cells and their infiltration by T cells, B cells, and macrophages<sup>[54]</sup>. Vitamin D has been shown to have immunomodulatory properties as well. Many immunomodulatory diseases such as rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, and inflammatory bowel disease have been reported to be associated with vitamin D deficiency<sup>[55,56]</sup>. Type 1 DM is also said to be related to vitamin D deficiency<sup>[57]</sup>. As VDR are expressed in human T and B lymphocytes, vitamin D is thought to modify the Th1/Th2 cytokine profile<sup>[58]</sup>. In addition, vitamin D is also thought to be associated with the immune system *via* its inhibition of lymphocyte proliferation<sup>[59]</sup>. Non-obese diabetic (NOD) mice with vitamin D deficiency showed an increased incidence and severity of diabetes<sup>[60]</sup>. Using 1,25(OH)<sub>2</sub>D reduced the manifestation of diabetes in NOD mice by decreasing the number of effector T cells<sup>[61,62]</sup>. Another study reported that 1,25(OH)<sub>2</sub>D also counteracted cytokine-induced expression of Fas, which regulates cell death in human islet cells<sup>[63]</sup>.

The relationship between sunlight exposure and the incidence of type 1 DM has been reported<sup>[64]</sup>. One study showed that providing vitamin D supplements to infants in North Europe, where daylight hours are shorter than in other countries, decreased the risk for new-onset type 1 DM<sup>[65]</sup>. Although children suspected of having rickets during the study period had a relative risk (RR) of 3.0 (1.0-9.0) for type 1 DM, children who had taken 2000 IU vitamin D daily had a RR = 0.22 (0.05-0.89). Some studies were designed to clarify the effect of vitamin D on the preservation of  $\beta$ -cell function after the onset of type 1 DM<sup>[66]</sup>. Two studies found no significant effects of administration on vitamin D in protecting  $\beta$ -cell function<sup>[67,68]</sup>. However, another study reported significant effects of vitamin D administration on maintaining  $\beta$ -cell function after the development of

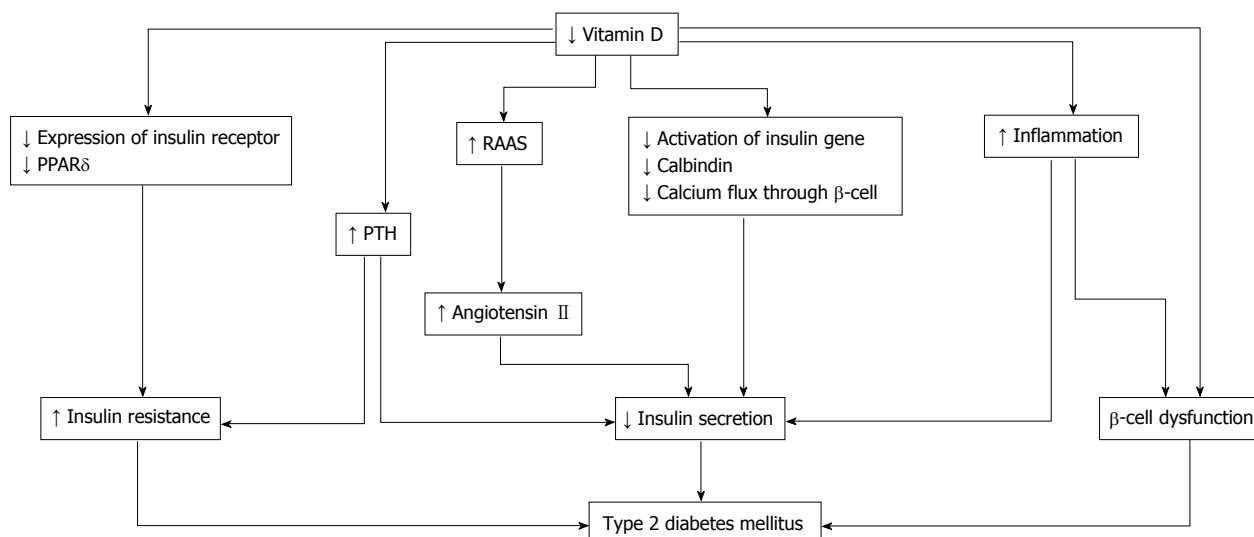
type 1 DM. Thirty-eight patients with new-onset type 1 DM were randomly assigned to receive daily oral therapy with cholecalciferol, 2000 IU, or placebo<sup>[69]</sup>. The cumulative incidence of progression to undetectable ( $\leq$  0.1 ng/mL) fasting C-peptide and stimulated C-peptide levels was lower in the cholecalciferol group than in the placebo group. In another study, alfalcidol (0.25  $\mu$ g/d) preserved  $\beta$ -cell function in children with newly diagnosed type 1 DM<sup>[70]</sup>. Further studies are needed to clarify whether the administration of 25(OH)D or 1,25(OH)<sub>2</sub>D can inhibit the onset of type 1 DM.

### Type 2 DM

As VDRs in pancreatic  $\beta$ -cells play an important role in the progression of type 2 DM<sup>[71]</sup>, vitamin D deficiency is related to insulin secretion, insulin resistance, and  $\beta$ -cell dysfunction in the pancreas<sup>[72]</sup> (Figure 2). The secretion of pancreatic insulin is inhibited by vitamin D deficiency in the diabetic animal model<sup>[73,74]</sup>. Administration of vitamin D restores glucose-stimulated insulin secretion and promotes  $\beta$ -cell survival by modulating the generation and effects of cytokines<sup>[75,76]</sup>. Insulin secretion is also influenced by calcium concentration and flux through the  $\beta$ -cells<sup>[77]</sup>. Vitamin D regulates the function of calbindin, a systolic calcium-binding protein found in pancreatic  $\beta$ -cells, and acts as a modulator of depolarization-stimulated insulin secretion *via* regulation of intracellular calcium<sup>[78]</sup>. PTH, which has its concentration regulated by vitamin D, is associated with insulin synthesis and secretion in the pancreas<sup>[79]</sup>.

Insulin sensitivity is also associated with vitamin D. By stimulating the expression of insulin receptors, vitamin D regulates insulin sensitivity<sup>[80,81]</sup>. In addition, vitamin D enhances insulin sensitivity by promoting the expression of peroxisome proliferator-activated receptor (PPAR) delta, which is a widely expressed member of the PPAR family of nuclear receptor fatty acid sensors and regulates fatty acids in skeletal muscle and adipose tissue<sup>[82]</sup>. Intracellular calcium is a key factor of peripheral insulin resistance *via* an impaired signal transduction pathway leading to decreased glucose transporter activity<sup>[83,84]</sup>.

The indirect effect of vitamin D is exerted by regulating calcium flux through the cell membrane and intracellular calcium. While low vitamin D induces secondary hyperparathyroidism, increased PTH levels are also associated with diabetes. A recent observational study of 494 women undergoing serial metabolic characterization revealed that hypovitamin D levels with increased PTH levels were an independent predictor of  $\beta$ -cell dysfunction, insulin resistance, and glycemia<sup>[85]</sup>. Vitamin D affects insulin resistance through the RAAS. One animal study demonstrated that vitamin D negatively regulated expression of renin genes in a mice model<sup>[86]</sup>. Furthermore, low levels of 1,25(OH)<sub>2</sub>D increased renal renin production and activated the RAAS system in an animal model<sup>[87]</sup>. Finally, angiotensin II inhibited the action of insulin in vascular and skeletal muscle tissues, leading to impaired glucose uptake<sup>[88]</sup>.



**Figure 2** Putative scheme of effect of vitamin D on type 2 diabetes mellitus. PPAR: Peroxisome proliferator-activated receptor; PTH: Parathyroid hormone; RAAS: Renin-angiotensin-aldosterone system.

Systemic inflammation has an important role in insulin resistance and cardiovascular events in patients with type 2 DM<sup>[89]</sup>. As  $\beta$ -cells in the pancreas are affected *via* cytokine-induced apoptosis, high levels of inflammation cause worsening glycemic control. Vitamin D could decrease the effects of systemic inflammation and protect against  $\beta$ -cell cytokine-induced apoptosis by directly modulating the expression and activity of cytokines, as has been shown in animal models<sup>[90]</sup>. In patients with type 2 DM, incubation of isolated monocytes with 1,25(OH)<sub>2</sub>D decreased the expression of inflammatory cytokines affecting insulin resistance, such as interleukin (IL)-1, IL-6, and tumor necrosis factor- $\alpha$ <sup>[91]</sup>.

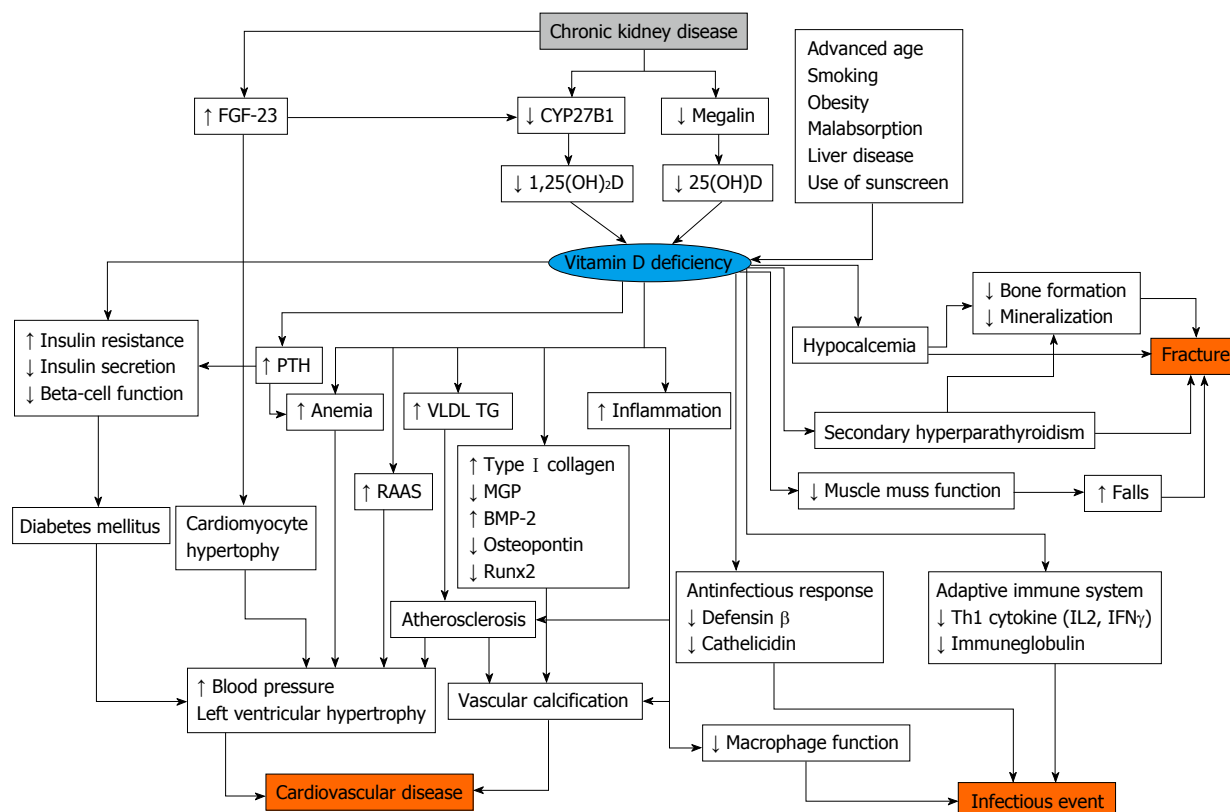
A prospective cohort study designed in the United Kingdom showed that baseline 25(OH)D concentrations in patients without diabetes were inversely related with the risk for hyperglycemia and insulin resistance at 10 years of follow-up visits<sup>[92]</sup>. Moreover, a similar study reported that low 25(OH)D levels were a risk factor for type 2 DM<sup>[93]</sup>. This prospective, cohort study was conducted over 29 years among 9841 subjects without diabetes. Lower vitamin D levels were a risk factor for incident type 2 DM. However, a recent Mendelian randomization approach study found that low 25(OH)D levels were not genetically associated with the risk for type 2 DM<sup>[94]</sup>. This result suggests that the association between 25(OH)D concentrations and type 2 DM is not causal. A meta-analysis of 16 studies reported that the odds ratio for type 2 DM was 1.5 (1.33-1.70) for the bottom vs top quartile of 25(OH)D levels<sup>[95]</sup>. Numerous randomized controlled studies have investigated whether vitamin D supplementation influences glycemic homeostasis<sup>[96,97]</sup>. As described above, vitamin D is thought to improve insulin resistance and promote insulin secretion. Therefore, clinical trials often use outcomes such as homeostasis model assessment of insulin resistance, fasting plasma glucose levels, and hemoglobin A1c

levels. Some clinical trials have assessed the combined effects of vitamin D and calcium supplementation on glucose homeostasis of patients with diabetes<sup>[98,99]</sup> and without diabetes<sup>[100]</sup>. These studies suggest that vitamin D plus adequate calcium levels might be needed for an improvement in glycemic status. However, a recent meta-analysis concluded that vitamin D supplementation given to address concerns with glycemic control and insulin resistance in patients with diabetes is not recommended, although the doses of vitamin D supplementation may not have been optimal; almost all of the included trials used vitamin D doses of at least 2000 IU/d<sup>[101]</sup>. Because most trials focused on glycemic status and insulin resistance over short durations (12 mo or less), we should await the results of ongoing trials with longer follow-up periods to provide new evidence regarding the potential role of vitamin D supplementation in type 2 DM<sup>[102]</sup>.

One study was designed to examine the protective effect of vitamin D against the development of type 2 DM<sup>[103]</sup>. A total of 2447 older people (mean age, 77 years) were allocated to 800 IU daily vitamin D<sub>3</sub> and 1000 mg calcium both, or placebo for 24-62 mo. Vitamin D in combination with calcium was not able to prevent the development of diabetes or an increase in the need for medication in patients with diabetes. The Women's Health Initiative Calcium/Vitamin D Study, a randomized, placebo-controlled trial of 33951 postmenopausal women, followed participants receiving 1000 mg elemental calcium plus 400 IU of vitamin D<sub>3</sub> daily, or placebo for 7 years. Calcium plus vitamin D<sub>3</sub> supplementation did not reduce the risk for developing diabetes over 7 years<sup>[104]</sup>. These results suggest that vitamin D supplementation at doses of 400 to 800 IU/d, with or without calcium, does not prevent new-onset type 2 DM.

Although at this time, supplementation with vitamin D has not been shown to improve glycemic control or





**Figure 3 Vitamin D deficiency and cardiovascular disease.** FGF-23: Fibroblast growth factor-23; PPAR: Peroxisome proliferator-activated receptor; PTH: Parathyroid hormone; RAAS: Renin-angiotensin-aldosterone system; VLDL: Very low density lipoprotein; TG: Triglycerides; IL: Interleukin; IFN: Interferon; MGP: Matrix gla protein; BMP: Bone morphogenetic protein.

prevent incident type 2 DM, clinical trials with sufficient sample size, study periods, and optimal doses of vitamin D supplementation are still needed. In addition, it is important that future studies of vitamin D use primary outcomes, such as all-cause mortality and cardiovascular disease, as endpoints.

## VITAMIN D AND CKD

One study revealed that paricalcitol diminished residual albuminuria in patients with diabetic nephropathy<sup>[105]</sup>. In this study, patients were randomly assigned (1:1:1) to receive placebo, 1 µg/d paricalcitol, or 2 µg/d paricalcitol for 24 wk to investigate the effect on mean urinary albumin-to-creatinine ratio (UACR). Patients receiving 2 µg paricalcitol showed a nearly sustained reduction in UACR, ranging from -18% to -28% ( $P = 0.014$  vs placebo). However, few trials have used a vitamin D receptor antagonist (VDRA) for patients with diabetes, and none has a sufficient number of patients or follow-up period. The effect of vitamin D<sub>3</sub> and VDRA on hard outcomes, such as progression of diabetes, cardiovascular disease, and all-cause mortality, requires larger and longer-term trials.

Some studies indicate that 1,25(OH)<sub>2</sub>D levels decrease in patients with CKD<sup>[106]</sup>. There are the several theories about the pathogenesis of vitamin D deficiency in CKD. Megalin, which is present in endocytic receptors in proximal tubule cells, is involved in the reab-

sorption of DBP from glomerular ultrafiltrates<sup>[107]</sup>. In addition, megalin also mediates the subsequent intracellular conversion of 25(OH)D to its active form. As kidney function declines, megalin expression in the proximal tubule decreases<sup>[108]</sup>. Megalin function is also attenuated with reduced kidney function, because of damages from low molecular weight proteinuria. The activity of CYP27B1 is also associated with decreasing kidney function<sup>[109]</sup>. As FGF-23 reduces expression of cotransporters NaPi- II a and NaPi- II c, of the brush border in the proximal tubules, these mechanisms inhibit phosphorus absorption and CYP27B1 activity.

In addition to the decline of 1,25(OH)<sub>2</sub>D levels, 25(OH)D levels also decrease in patients with CKD. There are the several plausible mechanisms that explain the decreases in 25(OH)D. The complex of 25(OH)D and DBP leaks with proteinuria. Uptake of 25(OH)D decreases due to down-regulation of megalin levels. One study showed that 25(OH)D concentrations in patients with CKD were low<sup>[110]</sup>. The prevalence of vitamin D deficiency is 35% among about 4000 patients with CKD in the United States<sup>[111]</sup>.

There is some evidence that vitamin D status is associated with poor clinical outcomes in patients with CKD<sup>[112]</sup> (Figure 3). Low 25(OH)D levels are associated with all-cause mortality and cardiovascular disease in patients with CKD<sup>[113]</sup>. The risk for end stage renal disease is higher in patients with low vitamin D status. Among patients undergoing hemodialysis and peritoneal

dialysis, low 25(OH)D levels are also associated with cardiovascular disease<sup>[114]</sup>.

There is some evidence regarding restitution of vitamin D in patients with CKD<sup>[115,116]</sup> and as well as in patients undergoing dialysis<sup>[117,118]</sup>. As previously described, patients with kidney failure usually have insufficient 1,25(OH)<sub>2</sub>D levels, and a VDRA is used for these patients. One study revealed that paricalcitol diminished albuminuria in patients with diabetic nephropathy<sup>[105]</sup>. In the VITAL study, which was designed to compare the effectiveness between paricalcitol and placebo, the paricalcitol group showed a decreased UACR of -16% compared with placebo<sup>[105]</sup>. However, as of yet, no other studies have investigated the effectiveness of vitamin D supplementation for protection of kidney function; thus, future studies are needed. Another study showed that paricalcitol led to decreases in levels of brain natriuretic peptide (BNP) in patients with CKD<sup>[119]</sup>. On the other hand, a recent study reported that treatment with paricalcitol did not improve left ventricular mass and function in patients with CKD<sup>[120]</sup>. There is controversial evidence regarding the role of VDRA to cardiovascular disease and surrogate makers. It is thought that as 1,25(OH)<sub>2</sub>D inhibits activation of the RAAS, it leads to organ protection<sup>[121]</sup>. In addition, there is some evidence regarding VDRAs in patients undergoing hemodialysis. A retrospective cohort study showed that VDRA users had a lower mortality rate than non-VDRA users<sup>[122]</sup>. However, the Dialysis Outcomes and Practice Patterns Study revealed that taking vitamin D agents did not improve clinical outcome in patients undergoing dialysis. In addition, a recent study reported that pharmacological doses of alfacalcidol were associated with accelerated progression of aortic stiffness in patients undergoing hemodialysis<sup>[123]</sup>. To date, various discussions have taken place regarding the use of VDRA in patients undergoing dialysis, but adequate clinical studies are needed before any recommendations can be made.

According to the Kidney Disease Improving Global Outcomes guidelines, 25(OH)D levels should be determined in patients with CKD stage 3-5, and if levels are low, physicians should consider vitamin D supplementation<sup>[124]</sup>. Low 25(OH)D levels are associated with all-cause mortality and cardiovascular disease in patients with CKD as well as in patients undergoing dialysis<sup>[125]</sup>. Another study showed that among these patient groups, those with low levels of 25(OH)D and high levels of FGF-23 have worse outcomes<sup>[38]</sup>. However, there is not sufficient evidence regarding vitamin D supplementation for patients with CKD and those undergoing dialysis<sup>[126]</sup>. Although studies have reported that cholecalciferol decreases albuminuria<sup>[127,128]</sup> and improves PTH levels<sup>[129]</sup> in patients with CKD, there is no study with set clinical outcomes such as all-cause mortality or cardiovascular disease. In patients undergoing dialysis, cholecalciferol decreases BNP levels and reduces left ventricular hypertrophy<sup>[130]</sup>. As VDRAs increase calcium and phosphorus levels in patients undergoing dialysis, it is usually recommended that physicians only need to monitor

calcium and phosphorus levels when using a VDRA<sup>[131]</sup>. On the other hand, vitamin D<sub>3</sub>, such as cholecalciferol, does not increase calcium and phosphorus levels<sup>[132,133]</sup>. As with patients with CKD, there is no evidence with hard endpoints regarding the use of vitamin D<sub>3</sub> supplementation in patients undergoing hemodialysis.

## CONCLUSION

Emerging evidence is accumulating on the important role of vitamin D in the pathogenesis of diabetes and CKD. Many prospective studies have shown associations between vitamin D status and chronic disease, including diabetes and CKD. However, there are contradictory findings regarding whether restitution of normal vitamin D levels modifies the occurrence or clinical course of these diseases. Although there is a concern that vitamin D may be a surrogate marker for poor health status, further well-designed clinical trials are needed in this area.

## REFERENCES

- 1 **Narayan KM**, Boyle JP, Geiss LS, Saaddine JB, Thompson TJ. Impact of recent increase in incidence on future diabetes burden: U.S., 2005-2050. *Diabetes Care* 2006; **29**: 2114-2116 [PMID: 16936162 DOI: 10.2337/dc06-1136]
- 2 **Ortiz A**, Covic A, Fliser D, Fouque D, Goldsmith D, Kanbay M, Mallamaci F, Massy ZA, Rossignol P, Vanholder R, Wiecek A, Zoccali C, London GM. Epidemiology, contributors to, and clinical trials of mortality risk in chronic kidney failure. *Lancet* 2014; **383**: 1831-1843 [PMID: 24856028 DOI: 10.1016/S0140-6736(14)60384-6]
- 3 **Holick MF**. High prevalence of vitamin D inadequacy and implications for health. *Mayo Clin Proc* 2006; **81**: 353-373 [PMID: 16529140 DOI: 10.4065/81.3.353]
- 4 **Husmoen LL**, Thuesen BH, Fenger M, Jørgensen T, Glümer C, Svensson J, Ovesen L, Witte DR, Linneberg A. Serum 25(OH)D and type 2 diabetes association in a general population: a prospective study. *Diabetes Care* 2012; **35**: 1695-1700 [PMID: 22688545 DOI: 10.2337/dc11-1309]
- 5 **George PS**, Pearson ER, Witham MD. Effect of vitamin D supplementation on glycaemic control and insulin resistance: a systematic review and meta-analysis. *Diabet Med* 2012; **29**: e142-e150 [PMID: 22486204 DOI: 10.1111/j.1464-5491.2012.03672.x]
- 6 **Holick MF**. Resurrection of vitamin D deficiency and rickets. *J Clin Invest* 2006; **116**: 2062-2072 [PMID: 16886050 DOI: 10.1172/JCI29449]
- 7 **Holick MF**. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. *Am J Clin Nutr* 2004; **80**: 1678S-1688S [PMID: 15585788]
- 8 **Christensen EI**, Willnow TE. Essential role of megalin in renal proximal tubule for vitamin homeostasis. *J Am Soc Nephrol* 1999; **10**: 2224-2236 [PMID: 10505701]
- 9 **Verroust PJ**, Birn H, Nielsen R, Kozyraki R, Christensen EI. The tandem endocytic receptors megalin and cubilin are important proteins in renal pathology. *Kidney Int* 2002; **62**: 745-756 [PMID: 12164855 DOI: 10.1046/j.1523-1755.2002.00501.x]
- 10 **Dusso AS**. Kidney disease and vitamin D levels: 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, and VDR activation. *Kidney Int Suppl* (2011) 2011; **1**: 136-141 [PMID: 25018912 DOI: 10.1038/kisup.2011.30]
- 11 **Silver J**, Naveh-Many T. FGF-23 and secondary hyperparathyroidism in chronic kidney disease. *Nat Rev Nephrol* 2013; **9**: 641-649 [PMID: 23877588 DOI: 10.1038/nrneph.2013.147]
- 12 **Campbell MJ**, Adorini L. The vitamin D receptor as a therapeutic

- target. *Expert Opin Ther Targets* 2006; **10**: 735-748 [PMID: 16981830 DOI: 10.1517/14728222.10.5.735]
- 13 **Segersten U**, Correa P, Hewison M, Hellman P, Dralle H, Carling T, Akerström G, Westin G. 25-hydroxyvitamin D(3)-1 $\alpha$ -hydroxylase expression in normal and pathological parathyroid glands. *J Clin Endocrinol Metab* 2002; **87**: 2967-2972 [PMID: 12050281]
  - 14 **van Driel M**, Koedam M, Buurman CJ, Hewison M, Chiba H, Uitterlinden AG, Pols HA, van Leeuwen JP. Evidence for auto/paracrine actions of vitamin D in bone: 1 $\alpha$ -hydroxylase expression and activity in human bone cells. *FASEB J* 2006; **20**: 2417-2419 [PMID: 17023519 DOI: 10.1096/fj.06-6374fje]
  - 15 **Krajisnik T**, Björklund P, Marsell R, Ljunggren O, Akerström G, Jonsson KB, Westin G, Larsson TE. Fibroblast growth factor-23 regulates parathyroid hormone and 1 $\alpha$ -hydroxylase expression in cultured bovine parathyroid cells. *J Endocrinol* 2007; **195**: 125-131 [PMID: 17911404 DOI: 10.1677/JOE-07-0267]
  - 16 Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium 2011 Dietary Reference Intakes for Calcium and Vitamin D. Washington (DC): National Academies Press, 2011
  - 17 **Buchebner D**, McGuigan F, Gerdhem P, Malm J, Ridderstråle M, Akesson K. Vitamin D insufficiency over 5 years is associated with increased fracture risk-an observational cohort study of elderly women. *Osteoporos Int* 2014; **25**: 2767-2775 [PMID: 25116384 DOI: 10.1007/s00198-014-2823-1]
  - 18 **Ensrud KE**, Taylor BC, Paudel ML, Cauley JA, Cawthon PM, Cummings SR, Fink HA, Barrett-Connor E, Zmuda JM, Shikany JM, Orwoll ES. Serum 25-hydroxyvitamin D levels and rate of hip bone loss in older men. *J Clin Endocrinol Metab* 2009; **94**: 2773-2780 [PMID: 19454586 DOI: 10.1210/jc.2008-2786]
  - 19 **Holick MF**, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2011; **96**: 1911-1930 [PMID: 21646368 DOI: 10.1210/jc.2011-0385]
  - 20 **DeLuca HF**. Overview of general physiologic features and functions of vitamin D. *Am J Clin Nutr* 2004; **80**: 1689S-1696S [PMID: 15585789]
  - 21 **Malabanan A**, Veronikis IE, Holick MF. Redefining vitamin D insufficiency. *Lancet* 1998; **351**: 805-806 [PMID: 9519960 DOI: 10.1016/S0140-6736(05)78933-9]
  - 22 **Bischoff-Ferrari HA**, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *Am J Clin Nutr* 2006; **84**: 18-28 [PMID: 16825677]
  - 23 **Gordon CM**, DePeter KC, Feldman HA, Grace E, Emans SJ. Prevalence of vitamin D deficiency among healthy adolescents. *Arch Pediatr Adolesc Med* 2004; **158**: 531-537 [PMID: 15184215 DOI: 10.1001/archpedi.158.6.531]
  - 24 **Sullivan SS**, Rosen CJ, Halteman WA, Chen TC, Holick MF. Adolescent girls in Maine are at risk for vitamin D insufficiency. *J Am Diet Assoc* 2005; **105**: 971-974 [PMID: 15942551 DOI: 10.1016/j.jada.2005.03.002]
  - 25 **Hypönen E**, Power C. Hypovitaminosis D in British adults at age 45 y: nationwide cohort study of dietary and lifestyle predictors. *Am J Clin Nutr* 2007; **85**: 860-868 [PMID: 17344510]
  - 26 **Jacques PF**, Felson DT, Tucker KL, Mahnken B, Wilson PW, Rosenberg IH, Rush D. Plasma 25-hydroxyvitamin D and its determinants in an elderly population sample. *Am J Clin Nutr* 1997; **66**: 929-936 [PMID: 9322570]
  - 27 **Lips P**. Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. *Endocr Rev* 2001; **22**: 477-501 [PMID: 11493580 DOI: 10.1210/edrv.22.4.0437]
  - 28 **Holick MF**, Siris ES, Binkley N, Beard MK, Khan A, Katzner JT, Petruschke RA, Chen E, de Papp AE. Prevalence of Vitamin D inadequacy among postmenopausal North American women receiving osteoporosis therapy. *J Clin Endocrinol Metab* 2005; **90**: 3215-3224 [PMID: 15797954 DOI: 10.1210/jc.2004-2364]
  - 29 **Mithal A**, Wahl DA, Bonjour JP, Burckhardt P, Dawson-Hughes B, Eisman JA, El-Hajj Fuleihan G, Josse RG, Lips P, Morales-Torres J. Global vitamin D status and determinants of hypovitaminosis D. *Osteoporos Int* 2009; **20**: 1807-1820 [PMID: 19543765 DOI: 10.1007/s00198-009-0954-6]
  - 30 **Chonchol M**, Scragg R. 25-Hydroxyvitamin D, insulin resistance, and kidney function in the Third National Health and Nutrition Examination Survey. *Kidney Int* 2007; **71**: 134-139 [PMID: 17082756]
  - 31 **Tanaka H**, Hamano T, Fujii N, Tomida K, Matsui I, Mikami S, Nagasawa Y, Ito T, Moriyama T, Horio M, Imai E, Isaka Y, Rakugi H. The impact of diabetes mellitus on vitamin D metabolism in predialysis patients. *Bone* 2009; **45**: 949-955 [PMID: 19631779 DOI: 10.1016/j.bone.2009.07.016]
  - 32 **Powe CE**, Evans MK, Wenger J, Zonderman AB, Berg AH, Nalls M, Tamez H, Zhang D, Bhan I, Karumanchi SA, Powe NR, Thadhani R. Vitamin D-binding protein and vitamin D status of black Americans and white Americans. *N Engl J Med* 2013; **369**: 1991-2000 [PMID: 24256378 DOI: 10.1056/NEJMoa1306357]
  - 33 **Kohli NR**, Van Valkengoed IG, Nicolaou M, Brewster LM, Van Der A DL, Stronks K, Snijder MB. Vitamin D status partly explains ethnic differences in blood pressure: the 'Surinamese in the Netherlands: study on ethnicity and health'. *J Hypertens* 2012; **30**: 1581-1587 [PMID: 22595957 DOI: 10.1097/HJH.0b013e328354cd2c]
  - 34 **Holick MF**. Vitamin D deficiency. *N Engl J Med* 2007; **357**: 266-281 [PMID: 17634462]
  - 35 **Arnaud J**, Constans J. Affinity differences for vitamin D metabolites associated with the genetic isoforms of the human serum carrier protein (DBP). *Hum Genet* 1993; **92**: 183-188 [PMID: 8370586]
  - 36 **Pittas AG**, Chung M, Trikalinos T, Mitri J, Brendel M, Patel K, Lichtenstein AH, Lau J, Balk EM. Systematic review: Vitamin D and cardiometabolic outcomes. *Ann Intern Med* 2010; **152**: 307-314 [PMID: 20194237 DOI: 10.7326/0003-4819-152-5-201003020-00009]
  - 37 **Schöttker B**, Jorde R, Peasey A, Thorand B, Jansen EH, Groot LD, Streppel M, Gardiner J, Ordóñez-Mena JM, Perna L, Wilsgaard T, Rathmann W, Feskens E, Kampman E, Siganos G, Njølstad I, Mathiesen EB, Kubínová R, Paják A, Topor-Madry R, Tamosiunas A, Hughes M, Kee F, Bobak M, Trichopoulos A, Boffetta P, Brenner H. Vitamin D and mortality: meta-analysis of individual participant data from a large consortium of cohort studies from Europe and the United States. *BMJ* 2014; **348**: g3656 [PMID: 24938302 DOI: 10.1136/bmj.g3656]
  - 38 **Chonchol M**, Greene T, Zhang Y, Hoofnagle AN, Cheung AK. Low Vitamin D and High Fibroblast Growth Factor 23 Serum Levels Associate with Infectious and Cardiac Deaths in the HEMO Study. *J Am Soc Nephrol* 2016; **27**: 227-237 [PMID: 25971439]
  - 39 **Autier P**, Boniol M, Pizot C, Mullie P. Vitamin D status and ill health: a systematic review. *Lancet Diabetes Endocrinol* 2014; **2**: 76-89 [PMID: 24622671 DOI: 10.1016/S2213-8587(13)70165-7]
  - 40 **Zittermann A**. Vitamin D and disease prevention with special reference to cardiovascular disease. *Prog Biophys Mol Biol* 2006; **92**: 39-48 [PMID: 16600341]
  - 41 **Brock KE**, Ke L, Tseng M, Clemson L, Koo FK, Jang H, Seibel MJ, Mpofu E, Fraser DR, Mason RS. Vitamin D status is associated with sun exposure, vitamin D and calcium intake, acculturation and attitudes in immigrant East Asian women living in Sydney. *J Steroid Biochem Mol Biol* 2013; **136**: 214-217 [PMID: 23262263 DOI: 10.1016/j.jsbmb.2012.12.005]
  - 42 **Krause R**, Bühring M, Hopfenmüller W, Holick MF, Sharma AM. Ultraviolet B and blood pressure. *Lancet* 1998; **352**: 709-710 [PMID: 9728997]
  - 43 **Forman JP**, Curhan GC, Taylor EN. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension among young women. *Hypertension* 2008; **52**: 828-832 [PMID: 18838623 DOI: 10.1161/HYPERTENSIONAHA.108.117630]
  - 44 **Vimalaswaran KS**, Cavadin A, Berry DJ, LifeLines Cohort Study investigators, Jorde R, Dieffenbach AK, Lu C, Alves AC, Heerspink

- HJ, Tikkanen E, Eriksson J, Wong A, Mangino M, Jablonski KA, Nolte IM, Houston DK, Ahluwalia TS, van der Most PJ, Pasko D, Zgaga L, Thiering E, Vitart V, Fraser RM, Huffman JE, de Boer RA, Schöttker B, Saum KU, McCarthy MI, Dupuis J, Herzig KH, Sebert S, Pouta A, Laitinen J, Kleber ME, Navis G, Lorentzon M, Jameson K, Arden N, Cooper JA, Acharya J, Hardy R, Raitakari O, Ripatti S, Billings LK, Lahti J, Osmond C, Penninx BW, Rejnmark L, Lohman KK, Paternoster L, Stolk RP, Hernandez DG, Byberg L, Hagström E, Melhus H, Ingelsson E, Mellström D, Ljunggren O, Tzoulaki I, McLachlan S, Theodoratou E, Tiesler CM, Julia A, Navarro P, Wright AF, Polasek O; International Consortium for Blood Pressure (ICBP); Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium; Global Blood Pressure Genetics (Global BPgen) consortium; Caroline Hayward, Wilson JF, Rudan I, Salomaa V, Heinrich J, Campbell H, Price JF, Karlsson M, Lind L, Michaëlsson K, Bandinelli S, Frayling TM, Hartman CA, Sørensen TI, Kritchevsky SB, Langdahl BL, Eriksson JG, Florez JC, Spector TD, Lehtimäki T, Kuh D, Humphries SE, Cooper C, Ohlsson C, März W, de Borst MH, Kumari M, Kivimäki M, Wang TJ, Power C, Brenner H, Grimmes G, van der Harst P, Snieder H, Hingorani AD, Pilz S, Whittaker JC, Jarvelin MR, Hyppönen E. Association of vitamin D status with arterial blood pressure and hypertension risk: a mendelian randomisation study. *Lancet Diabetes Endocrinol* 2014; **2**: 719-729 [PMID: 24974252 DOI: 10.1016/S2213-8587(14)70113-5]
- 45 **Kilkinen A**, Knekt P, Aro A, Rissanen H, Marniemi J, Heliövaara M, Impivaara O, Reunanen A. Vitamin D status and the risk of cardiovascular disease death. *Am J Epidemiol* 2009; **170**: 1032-1039 [PMID: 19762371 DOI: 10.1093/aje/kwp227]
- 46 **Giovannucci E**, Liu Y, Hollis BW, Rimm EB. 25-hydroxyvitamin D and risk of myocardial infarction in men: a prospective study. *Arch Intern Med* 2008; **168**: 1174-1180 [PMID: 18541825 DOI: 10.1001/archinte.168.11.1174]
- 47 **di Giuseppe R**, Buijsse B, Hirche F, Wirth J, Arregui M, Westphal S, Isermann B, Hense HW, Dierkes J, Boeing H, Stangl GI, Weikert C. Plasma fibroblast growth factor 23, parathyroid hormone, 25-hydroxyvitamin D3, and risk of heart failure: a prospective, case-cohort study. *J Clin Endocrinol Metab* 2014; **99**: 947-955 [PMID: 24423292 DOI: 10.1210/jc.2013-2963]
- 48 **Brøndum-Jacobsen P**, Nordestgaard BG, Schnohr P, Benn M. 25-hydroxyvitamin D and symptomatic ischemic stroke: an original study and meta-analysis. *Ann Neurol* 2013; **73**: 38-47 [PMID: 23225498 DOI: 10.1002/ana.23738]
- 49 **Durup D**, Jørgensen HL, Christensen J, Tjønneland A, Olsen A, Halkjær J, Lind B, Heegaard AM, Schwarz P. A Reverse J-Shaped Association Between Serum 25-Hydroxyvitamin D and Cardiovascular Disease Mortality: The CopD Study. *J Clin Endocrinol Metab* 2015; **100**: 2339-2346 [PMID: 25710567 DOI: 10.1210/jc.2014-4551]
- 50 **Li YC**, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. *J Clin Invest* 2002; **110**: 229-238 [PMID: 12122115]
- 51 **Li YC**. Renoprotective effects of vitamin D analogs. *Kidney Int* 2010; **78**: 134-139 [PMID: 19471320 DOI: 10.1038/ki.2009.175]
- 52 **Andrukhova O**, Slavic S, Zeitz U, Riesen SC, Heppelmann MS, Ambrisko TD, Markovic M, Kuebler WM, Erben RG. Vitamin D is a regulator of endothelial nitric oxide synthase and arterial stiffness in mice. *Mol Endocrinol* 2014; **28**: 53-64 [PMID: 24284821 DOI: 10.1210/me.2013-1252]
- 53 **Nakai K**, Fujii H, Kono K, Goto S, Kitazawa R, Kitazawa S, Hirata M, Shinohara M, Fukagawa M, Nishi S. Vitamin D activates the Nrf2-Keap1 antioxidant pathway and ameliorates nephropathy in diabetic rats. *Am J Hypertens* 2014; **27**: 586-595 [PMID: 24025724 DOI: 10.1093/ajh/hpt160]
- 54 **Atkinson MA**, Eisenbarth GS. Type 1 diabetes: new perspectives on disease pathogenesis and treatment. *Lancet* 2001; **358**: 221-229 [PMID: 11476858 DOI: 10.1016/S0140-6736(01)05415-0]
- 55 **Lin CH**, Kadakia S, Frieri M. New insights into an autoimmune mechanism, pharmacological treatment and relationship between multiple sclerosis and inflammatory bowel disease. *Autoimmun Rev* 2014; **13**: 114-116 [PMID: 24129036 DOI: 10.1016/j.autrev.2013.09.011]
- 56 **Gatenby P**, Lucas R, Swaminathan A. Vitamin D deficiency and risk for rheumatic diseases: an update. *Curr Opin Rheumatol* 2013; **25**: 184-191 [PMID: 23370372 DOI: 10.1097/BOR.0b013e32835cfc16]
- 57 **Yang CY**, Leung PS, Adamopoulos IE, Gershwin ME. The implication of vitamin D and autoimmunity: a comprehensive review. *Clin Rev Allergy Immunol* 2013; **45**: 217-226 [PMID: 23359064 DOI: 10.1007/s12016-013-8361-3]
- 58 **Antico A**, Tampoia M, Tozzoli R, Bizzaro N. Can supplementation with vitamin D reduce the risk or modify the course of autoimmune diseases? A systematic review of the literature. *Autoimmun Rev* 2012; **12**: 127-136 [PMID: 22776787 DOI: 10.1016/j.autrev.2012.07.007]
- 59 **Arnsen Y**, Amital H, Shoenfeld Y. Vitamin D and autoimmunity: new aetiological and therapeutic considerations. *Ann Rheum Dis* 2007; **66**: 1137-1142 [PMID: 17557889 DOI: 10.1136/ard.2007.069-831]
- 60 **Zella JB**, McCarty LC, DeLuca HF. Oral administration of 1,25-dihydroxyvitamin D3 completely protects NOD mice from insulin-dependent diabetes mellitus. *Arch Biochem Biophys* 2003; **417**: 77-80 [PMID: 12921782 DOI: 10.1016/S0003-9861(03)00338-2]
- 61 **Zella JB**, DeLuca HF. Vitamin D and autoimmune diabetes. *J Cell Biochem* 2003; **88**: 216-222 [PMID: 12520517 DOI: 10.1002/jcb.10347]
- 62 **Takiishi T**, Ding L, Baeke F, Spagnuolo I, Sebastiani G, Laureys J, Verstuyf A, Carmeliet G, Dotta F, Van Belle TL, Gysemans CA, Mathieu C. Dietary supplementation with high doses of regular vitamin D3 safely reduces diabetes incidence in NOD mice when given early and long term. *Diabetes* 2014; **63**: 2026-2036 [PMID: 24550187 DOI: 10.2337/db13-1559]
- 63 **Riachy R**, Vandewalle B, Moerman E, Belaich S, Lukowiak B, Gmyr V, Muharram G, Kerr Conte J, Pattou F. 1,25-Dihydroxyvitamin D3 protects human pancreatic islets against cytokine-induced apoptosis via down-regulation of the Fas receptor. *Apoptosis* 2006; **11**: 151-159 [PMID: 16502254 DOI: 10.1007/s10495-006-3558-z]
- 64 Geographic patterns of childhood insulin-dependent diabetes mellitus. Diabetes Epidemiology Research International Group. *Diabetes* 1988; **37**: 1113-1119 [PMID: 3391346 DOI: 10.2337/diab.37.8.1113]
- 65 **Hyppönen E**, Läärä E, Reunanen A, Jarvelin MR, Virtanen SM. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. *Lancet* 2001; **358**: 1500-1503 [PMID: 11705562 DOI: 10.1016/S0140-6736(01)06580-1]
- 66 Vitamin D supplement in early childhood and risk for Type I (insulin-dependent) diabetes mellitus. The EURODIAB Substudy 2 Study Group. *Diabetologia* 1999; **42**: 51-54 [PMID: 10027578 DOI: 10.1007/s001250051112]
- 67 **Bizzarri C**, Pitocco D, Napoli N, Di Stasio E, Maggi D, Manfrini S, Suraci C, Cavallo MG, Cappa M, Ghirlanda G, Pozzilli P. No protective effect of calcitriol on beta-cell function in recent-onset type 1 diabetes: the IMDIAB XIII trial. *Diabetes Care* 2010; **33**: 1962-1963 [PMID: 20805274 DOI: 10.2337/dc10-0814]
- 68 **Walter M**, Kaupfer T, Adler K, Foersch J, Bonifacio E, Ziegler AG. No effect of the 1 $\alpha$ ,25-dihydroxyvitamin D3 on beta-cell residual function and insulin requirement in adults with new-onset type 1 diabetes. *Diabetes Care* 2010; **33**: 1443-1448 [PMID: 20357369 DOI: 10.2337/dc09-2297]
- 69 **Gabbay MA**, Sato MN, Finazzo C, Duarte AJ, Dib SA. Effect of cholecalciferol as adjunctive therapy with insulin on protective immunologic profile and decline of residual  $\beta$ -cell function in new-onset type 1 diabetes mellitus. *Arch Pediatr Adolesc Med* 2012; **166**: 601-607 [PMID: 22751874 DOI: 10.1001/archpediatrics.2012.164]
- 70 **Ataie-Jafari A**, Loke SC, Rahmat AB, Larijani B, Abbasi F, Leow MK, Yassin Z. A randomized placebo-controlled trial of alphacalcidol on the preservation of beta cell function in children



- with recent onset type 1 diabetes. *Clin Nutr* 2013; **32**: 911-917 [PMID: 23395257 DOI: 10.1016/j.clnu.2013.01.012]
- 71 **Palomer X**, González-Clemente JM, Blanco-Vaca F, Mauricio D. Role of vitamin D in the pathogenesis of type 2 diabetes mellitus. *Diabetes Obes Metab* 2008; **10**: 185-197 [PMID: 18269634 DOI: 10.1111/j.1463-1326.2007.00710.x]
  - 72 **Chiu KC**, Chu A, Go VL, Saad MF. Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. *Am J Clin Nutr* 2004; **79**: 820-825 [PMID: 15113720]
  - 73 **Norman AW**, Frankel JB, Heldt AM, Grodsky GM. Vitamin D deficiency inhibits pancreatic secretion of insulin. *Science* 1980; **209**: 823-825 [PMID: 6250216 DOI: 10.1126/science.6250216]
  - 74 **Cade C**, Norman AW. Vitamin D3 improves impaired glucose tolerance and insulin secretion in the vitamin D-deficient rat in vivo. *Endocrinology* 1986; **119**: 84-90 [PMID: 3013599 DOI: 10.1210/endo-119-1-84]
  - 75 **Cade C**, Norman AW. Rapid normalization/stimulation by 1,25-dihydroxyvitamin D3 of insulin secretion and glucose tolerance in the vitamin D-deficient rat. *Endocrinology* 1987; **120**: 1490-1497 [PMID: 3549262 DOI: 10.1210/endo-120-4-1490]
  - 76 **Clark SA**, Stumpf WE, Sar M. Effect of 1,25 dihydroxyvitamin D3 on insulin secretion. *Diabetes* 1981; **30**: 382-386 [PMID: 7014306 DOI: 10.2337/diab.30.5.382]
  - 77 **Wolden-Kirk H**, Overbergh L, Christesen HT, Brusgaard K, Mathieu C. Vitamin D and diabetes: its importance for beta cell and immune function. *Mol Cell Endocrinol* 2011; **347**: 106-120 [PMID: 21889571 DOI: 10.1016/j.mce.2011.08.016]
  - 78 **Kadowaki S**, Norman AW. Pancreatic vitamin D-dependent calcium binding protein: biochemical properties and response to vitamin D. *Arch Biochem Biophys* 1984; **233**: 228-236 [PMID: 6087742 DOI: 10.1016/0003-9861(84)90621-0]
  - 79 **Fadda GZ**, Akmal M, Lipson LG, Massry SG. Direct effect of parathyroid hormone on insulin secretion from pancreatic islets. *Am J Physiol* 1990; **258**: E975-E984 [PMID: 2193536]
  - 80 **Maestro B**, Molero S, Bajo S, Dávila N, Calle C. Transcriptional activation of the human insulin receptor gene by 1,25-dihydroxyvitamin D(3). *Cell Biochem Funct* 2002; **20**: 227-232 [PMID: 12125099 DOI: 10.1002/cbf.951]
  - 81 **Maestro B**, Dávila N, Carranza MC, Calle C. Identification of a Vitamin D response element in the human insulin receptor gene promoter. *J Steroid Biochem Mol Biol* 2003; **84**: 223-230 [PMID: 12711007 DOI: 10.1016/S0960-0760(03)00032-3]
  - 82 **Dunlop TW**, Väisänen S, Frank C, Molnár F, Sinkkonen L, Carlberg C. The human peroxisome proliferator-activated receptor delta gene is a primary target of 1alpha,25-dihydroxyvitamin D3 and its nuclear receptor. *J Mol Biol* 2005; **349**: 248-260 [PMID: 15890193 DOI: 10.1016/j.jmb.2005.03.060]
  - 83 **Zemel MB**. Nutritional and endocrine modulation of intracellular calcium: implications in obesity, insulin resistance and hypertension. *Mol Cell Biochem* 1998; **188**: 129-136 [PMID: 9823018 DOI: 10.1023/A:1006880708475]
  - 84 **Reusch JE**, Begum N, Sussman KE, Draznin B. Regulation of GLUT-4 phosphorylation by intracellular calcium in adipocytes. *Endocrinology* 1991; **129**: 3269-3273 [PMID: 1659526 DOI: 10.1210/endo-129-6-3269]
  - 85 **Kramer CK**, Swaminathan B, Hanley AJ, Connelly PW, Sermer M, Zinman B, Retnakaran R. Prospective associations of vitamin D status with  $\beta$ -cell function, insulin sensitivity, and glycemia: the impact of parathyroid hormone status. *Diabetes* 2014; **63**: 3868-3879 [PMID: 24875346 DOI: 10.2337/db14-0489]
  - 86 **Xiang W**, Kong J, Chen S, Cao LP, Qiao G, Zheng W, Liu W, Li X, Gardner DG, Li YC. Cardiac hypertrophy in vitamin D receptor knockout mice: role of the systemic and cardiac renin-angiotensin systems. *Am J Physiol Endocrinol Metab* 2005; **288**: E125-E132 [PMID: 15367398]
  - 87 **Kong J**, Qiao G, Zhang Z, Liu SQ, Li YC. Targeted vitamin D receptor expression in juxtaglomerular cells suppresses renin expression independent of parathyroid hormone and calcium. *Kidney Int* 2008; **74**: 1577-1581 [PMID: 19034301 DOI: 10.1038/ki.2008.452]
  - 88 **Cheng Q**, Boucher BJ, Leung PS. Modulation of hypovitaminosis D-induced islet dysfunction and insulin resistance through direct suppression of the pancreatic islet renin-angiotensin system in mice. *Diabetologia* 2013; **56**: 553-562 [PMID: 23250033 DOI: 10.1007/s00125-012-2801-0]
  - 89 **Pradhan A**. Obesity, metabolic syndrome, and type 2 diabetes: inflammatory basis of glucose metabolic disorders. *Nutr Rev* 2007; **65**: S152-S156 [PMID: 18240540]
  - 90 **Riachy R**, Vandewalle B, Kerr Conte J, Moerman E, Sacchetti P, Lukowiak B, Gmyr V, Bouckenoghe T, Dubois M, Pattou F. 1,25-dihydroxyvitamin D3 protects RINm5F and human islet cells against cytokine-induced apoptosis: implication of the antiapoptotic protein A20. *Endocrinology* 2002; **143**: 4809-4819 [PMID: 12446608]
  - 91 **Giulietti A**, van Etten E, Overbergh L, Stoffels K, Bouillon R, Mathieu C. Monocytes from type 2 diabetic patients have a pro-inflammatory profile. 1,25-Dihydroxyvitamin D(3) works as anti-inflammatory. *Diabetes Res Clin Pract* 2007; **77**: 47-57 [PMID: 17112620]
  - 92 **Forouhi NG**, Luan J, Cooper A, Boucher BJ, Wareham NJ. Baseline serum 25-hydroxy vitamin d is predictive of future glycemic status and insulin resistance: the Medical Research Council Ely Prospective Study 1990-2000. *Diabetes* 2008; **57**: 2619-2625 [PMID: 18591391 DOI: 10.2337/db08-0593]
  - 93 **Afzal S**, Bojesen SE, Nordestgaard BG. Low 25-hydroxyvitamin D and risk of type 2 diabetes: a prospective cohort study and metaanalysis. *Clin Chem* 2013; **59**: 381-391 [PMID: 23232064 DOI: 10.1373/clinchem.2012.193003]
  - 94 **Ye Z**, Sharp SJ, Burgess S, Scott RA, Imamura F, Langenberg C, Wareham NJ, Forouhi NG. Association between circulating 25-hydroxyvitamin D and incident type 2 diabetes: a mendelian randomisation study. *Lancet Diabetes Endocrinol* 2015; **3**: 35-42 [PMID: 25281353 DOI: 10.1016/S2213-8587(14)70184-6]
  - 95 **Parekh D**, Sarathi V, Shivane VK, Bandgar TR, Menon PS, Shah NS. Pilot study to evaluate the effect of short-term improvement in vitamin D status on glucose tolerance in patients with type 2 diabetes mellitus. *Endocr Pract* 2010; **16**: 600-608 [PMID: 20350923 DOI: 10.4158/EP09300.OR]
  - 96 **Harris SS**, Pittas AG, Palermo NJ. A randomized, placebo-controlled trial of vitamin D supplementation to improve glycaemia in overweight and obese African Americans. *Diabetes Obes Metab* 2012; **14**: 789-794 [PMID: 22486948 DOI: 10.1111/j.1463-1326.2012.01605.x]
  - 97 **Oosterwerff MM**, Eekhoff EM, Van Schoor NM, Boeke AJ, Nanayakkara P, Meijnen R, Knol DL, Kramer MH, Lips P. Effect of moderate-dose vitamin D supplementation on insulin sensitivity in vitamin D-deficient non-Western immigrants in the Netherlands: a randomized placebo-controlled trial. *Am J Clin Nutr* 2014; **100**: 152-160 [PMID: 24898240 DOI: 10.3945/ajcn.113.069260]
  - 98 **Mitri J**, Dawson-Hughes B, Hu FB, Pittas AG. Effects of vitamin D and calcium supplementation on pancreatic  $\beta$  cell function, insulin sensitivity, and glycemia in adults at high risk of diabetes: the Calcium and Vitamin D for Diabetes Mellitus (CaDDM) randomized controlled trial. *Am J Clin Nutr* 2011; **94**: 486-494 [PMID: 21715514 DOI: 10.3945/ajcn.111.011684]
  - 99 **Tabesh M**, Azadbakht L, Faghihimani E, Tabesh M, Esmailzadeh A. Effects of calcium-vitamin D co-supplementation on metabolic profiles in vitamin D insufficient people with type 2 diabetes: a randomised controlled clinical trial. *Diabetologia* 2014; **57**: 2038-2047 [PMID: 25005333 DOI: 10.1007/s00125-014-3313-x]
  - 100 **Pittas AG**, Harris SS, Stark PC, Dawson-Hughes B. The effects of calcium and vitamin D supplementation on blood glucose and markers of inflammation in nondiabetic adults. *Diabetes Care* 2007; **30**: 980-986 [PMID: 17277040 DOI: 10.2337/dc06-1994]
  - 101 **Seida JC**, Mitri J, Colmers IN, Majumdar SR, Davidson MB, Edwards AL, Hanley DA, Pittas AG, Tjosvold L, Johnson JA. Clinical review: Effect of vitamin D3 supplementation on improving glucose homeostasis and preventing diabetes: a systematic review and meta-analysis. *J Clin Endocrinol Metab* 2014; **99**: 3551-3560 [PMID: 25062463 DOI: 10.1210/jc.2014-2136]

- 102 **Manson JE**, Bassuk SS, Lee IM, Cook NR, Albert MA, Gordon D, Zaharris E, Macfadyen JG, Danielson E, Lin J, Zhang SM, Buring JE. The VITamin D and Omega-3 TriaL (VITAL): rationale and design of a large randomized controlled trial of vitamin D and marine omega-3 fatty acid supplements for the primary prevention of cancer and cardiovascular disease. *Contemp Clin Trials* 2012; **33**: 159-171 [PMID: 21986389 DOI: 10.1016/j.cct.2011.09.009]
- 103 **Avenell A**, Cook JA, MacLennan GS, McPherson GC. Vitamin D supplementation and type 2 diabetes: a substudy of a randomised placebo-controlled trial in older people (RECORD trial, ISRCTN 51647438). *Age Ageing* 2009; **38**: 606-609 [PMID: 19617604 DOI: 10.1093/ageing/afp109]
- 104 **de Boer IH**, Tinker LF, Connelly S, Curb JD, Howard BV, Kestenbaum B, Larson JC, Manson JE, Margolis KL, Siscovick DS, Weiss NS. Calcium plus vitamin D supplementation and the risk of incident diabetes in the Women's Health Initiative. *Diabetes Care* 2008; **31**: 701-707 [PMID: 18235052 DOI: 10.2337/dc07-1829]
- 105 **de Zeeuw D**, Agarwal R, Amdahl M, Audhya P, Coyne D, Garimella T, Parving HH, Pritchett Y, Remuzzi G, Ritz E, Andress D. Selective vitamin D receptor activation with paricalcitol for reduction of albuminuria in patients with type 2 diabetes (VITAL study): a randomised controlled trial. *Lancet* 2010; **376**: 1543-1551 [PMID: 21055801 DOI: 10.1016/S0140-6736(10)61032-X]
- 106 **Kendrick J**, Cheung AK, Kaufman JS, Greene T, Roberts WL, Smits G, Chonchol M. Associations of plasma 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D concentrations with death and progression to maintenance dialysis in patients with advanced kidney disease. *Am J Kidney Dis* 2012; **60**: 567-575 [PMID: 22621970 DOI: 10.1053/j.ajkd.2012.04.014]
- 107 **Saito A**, Pietromonaco S, Loo AK, Farquhar MG. Complete cloning and sequencing of rat gp330/"megalin," a distinctive member of the low density lipoprotein receptor gene family. *Proc Natl Acad Sci USA* 1994; **91**: 9725-9729 [PMID: 7937880 DOI: 10.1073/pnas.91.21.9725]
- 108 **Hosaka K**, Takeda T, Iino N, Hosojima M, Sato H, Kaseda R, Yamamoto K, Kobayashi A, Gejyo F, Saito A. Megalin and nonmuscle myosin heavy chain IIA interact with the adaptor protein Disabled-2 in proximal tubule cells. *Kidney Int* 2009; **75**: 1308-1315 [PMID: 19340093 DOI: 10.1038/ki.2009.85]
- 109 **Bouillon R**, Carmeliet G, Lieben L, Watanabe M, Perino A, Auwerx J, Schoonjans K, Verstuyf A. Vitamin D and energy homeostasis: of mice and men. *Nat Rev Endocrinol* 2014; **10**: 79-87 [PMID: 24247221 DOI: 10.1038/nrendo.2013.226]
- 110 **Pilz S**, Iodice S, Zittermann A, Grant WB, Gandini S. Vitamin D status and mortality risk in CKD: a meta-analysis of prospective studies. *Am J Kidney Dis* 2011; **58**: 374-382 [PMID: 21636193 DOI: 10.1053/j.ajkd.2011.03.020]
- 111 **Isakova T**, Wahl P, Vargas GS, Gutiérrez OM, Scialla J, Xie H, Appleby D, Nessel L, Bellovich K, Chen J, Hamm L, Gadegbeku C, Horwitz E, Townsend RR, Anderson CA, Lash JP, Hsu CY, Leonard MB, Wolf M. Fibroblast growth factor 23 is elevated before parathyroid hormone and phosphate in chronic kidney disease. *Kidney Int* 2011; **79**: 1370-1378 [PMID: 21389978 DOI: 10.1038/ki.2011.47]
- 112 **Mehrotra R**, Kermah DA, Salusky IB, Wolf MS, Thadhani RI, Chiu YW, Martins D, Adler SG, Norris KC. Chronic kidney disease, hypovitaminosis D, and mortality in the United States. *Kidney Int* 2009; **76**: 977-983 [PMID: 19657329 DOI: 10.1038/ki.2009.288]
- 113 **Navaneethan SD**, Schold JD, Arrigain S, Jolly SE, Jain A, Schreiber MJ, Simon JF, Srinivas TR, Nally JV. Low 25-hydroxyvitamin D levels and mortality in non-dialysis-dependent CKD. *Am J Kidney Dis* 2011; **58**: 536-543 [PMID: 21816525 DOI: 10.1053/j.ajkd.2011.04.028]
- 114 **Wang AY**, Lam CW, Sanderson JE, Wang M, Chan IH, Lui SF, Sea MM, Woo J. Serum 25-hydroxyvitamin D status and cardiovascular outcomes in chronic peritoneal dialysis patients: a 3-y prospective cohort study. *Am J Clin Nutr* 2008; **87**: 1631-1638 [PMID: 18541550]
- 115 **Kooienga L**, Fried L, Scragg R, Kendrick J, Smits G, Chonchol M. The effect of combined calcium and vitamin D3 supplementation on serum intact parathyroid hormone in moderate CKD. *Am J Kidney Dis* 2009; **53**: 408-416 [PMID: 19185400 DOI: 10.1053/j.ajkd.2008.09.020]
- 116 **Shroff R**, Wan M, Gullett A, Ledermann S, Shute R, Knott C, Wells D, Aitkenhead H, Manickavasagar B, van't Hoff W, Rees L. Ergocalciferol supplementation in children with CKD delays the onset of secondary hyperparathyroidism: a randomized trial. *Clin J Am Soc Nephrol* 2012; **7**: 216-223 [PMID: 22266572 DOI: 10.2215/CJN.04760511]
- 117 **Jean G**, Souberbielle JC, Chazot C. Monthly cholecalciferol administration in haemodialysis patients: a simple and efficient strategy for vitamin D supplementation. *Nephrol Dial Transplant* 2009; **24**: 3799-3805 [PMID: 19622574 DOI: 10.1093/ndt/gfp370]
- 118 **Armas LA**, Zena M, Lund R, Heaney RP. Calcium absorption response to cholecalciferol supplementation in hemodialysis. *Clin J Am Soc Nephrol* 2013; **8**: 1003-1008 [PMID: 23411428 DOI: 10.2215/CJN.08610812]
- 119 **Tamez H**, Zoccali C, Packham D, Wenger J, Bhan I, Appelbaum E, Pritchett Y, Chang Y, Agarwal R, Wanner C, Lloyd-Jones D, Cannata J, Thompson BT, Andress D, Zhang W, Singh B, Zehnder D, Pachika A, Manning WJ, Shah A, Solomon SD, Thadhani R. Vitamin D reduces left atrial volume in patients with left ventricular hypertrophy and chronic kidney disease. *Am Heart J* 2012; **164**: 902-909.e2 [PMID: 23194491 DOI: 10.1016/j.ahj.2012.09.018]
- 120 **Wang AY**, Fang F, Chan J, Wen YY, Qing S, Chan IH, Lo G, Lai KN, Lo WK, Lam CW, Yu CM. Effect of paricalcitol on left ventricular mass and function in CKD--the OPERA trial. *J Am Soc Nephrol* 2014; **25**: 175-186 [PMID: 24052631 DOI: 10.1681/ASN.2013010103]
- 121 **de Borst MH**, Vervloet MG, ter Wee PM, Navis G. Cross talk between the renin-angiotensin-aldosterone system and vitamin D-FGF-23-klotho in chronic kidney disease. *J Am Soc Nephrol* 2011; **22**: 1603-1609 [PMID: 21852584 DOI: 10.1681/ASN.2010121251]
- 122 **Teng M**, Wolf M, Ofsthun MN, Lazarus JM, Hernán MA, Camargo CA, Thadhani R. Activated injectable vitamin D and hemodialysis survival: a historical cohort study. *J Am Soc Nephrol* 2005; **16**: 1115-1125 [PMID: 15728786]
- 123 **Charitaki E**, Davenport A. Aortic pulse wave velocity in haemodialysis patients is associated with the prescription of active vitamin D analogues. *J Nephrol* 2014; **27**: 431-437 [PMID: 24473732 DOI: 10.1007/s40620-014-0040-9]
- 124 **Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Work Group**. KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD). *Kidney Int Suppl* 2009; **(113)**: S1-130 [PMID: 19644521 DOI: 10.1038/ki.2009.188]
- 125 **Wolf M**, Shah A, Gutierrez O, Ankers E, Monroy M, Tamez H, Steele D, Chang Y, Camargo CA, Tonelli M, Thadhani R. Vitamin D levels and early mortality among incident hemodialysis patients. *Kidney Int* 2007; **72**: 1004-1013 [PMID: 17687259]
- 126 **Kandula P**, Dobre M, Schold JD, Schreiber MJ, Mehrotra R, Navaneethan SD. Vitamin D supplementation in chronic kidney disease: a systematic review and meta-analysis of observational studies and randomized controlled trials. *Clin J Am Soc Nephrol* 2011; **6**: 50-62 [PMID: 20876671 DOI: 10.2215/CJN.03940510]
- 127 **Molina P**, Górriz JL, Molina MD, Peris A, Beltrán S, Kanter J, Escudero V, Romero R, Pallardó LM. The effect of cholecalciferol for lowering albuminuria in chronic kidney disease: a prospective controlled study. *Nephrol Dial Transplant* 2014; **29**: 97-109 [PMID: 23975842 DOI: 10.1093/ndt/gft360]
- 128 **Kim MJ**, Frankel AH, Donaldson M, Darch SJ, Pusey CD, Hill PD, Mayr M, Tam FW. Oral cholecalciferol decreases albuminuria and urinary TGF- $\beta$ 1 in patients with type 2 diabetic nephropathy on established renin-angiotensin-aldosterone system inhibition. *Kidney Int* 2011; **80**: 851-860 [PMID: 21832985 DOI: 10.1038/ki.2011.224]
- 129 **Alvarez JA**, Law J, Coakley KE, Zughaier SM, Hao L, Shahid Salles K, Wasse H, Gutiérrez OM, Ziegler TR, Tangpricha V. High-

- dose cholecalciferol reduces parathyroid hormone in patients with early chronic kidney disease: a pilot, randomized, double-blind, placebo-controlled trial. *Am J Clin Nutr* 2012; **96**: 672-679 [PMID: 22854402 DOI: 10.3945/ajcn.112.040642]
- 130 **Bucharies S**, Barberato SH, Stinghen AE, Gruber B, Piekala L, Dambiski AC, Custodio MR, Pecoits-Filho R. Impact of cholecalciferol treatment on biomarkers of inflammation and myocardial structure in hemodialysis patients without hyperparathyroidism. *J Ren Nutr* 2012; **22**: 284-291 [PMID: 21908203 DOI: 10.1053/j.jrn.2011.07.001]
  - 131 **Warn PA**, Sharp A, Morrissey G, Denning DW. Activity of aminocandin (IP960; HMR3270) compared with amphotericin B, itraconazole, caspofungin and micafungin in neutropenic murine models of disseminated infection caused by itraconazole-susceptible and -resistant strains of *Aspergillus fumigatus*. *Int J Antimicrob Agents* 2010; **35**: 146-151 [PMID: 20015618 DOI: 10.1016/j.ijantimicag.2009.09.029]
  - 132 **Armas LA**, Andukuri R, Barger-Lux J, Heaney RP, Lund R. 25-Hydroxyvitamin D response to cholecalciferol supplementation in hemodialysis. *Clin J Am Soc Nephrol* 2012; **7**: 1428-1434 [PMID: 22798536 DOI: 10.2215/CJN.12761211]
  - 133 **Massart A**, Debelle FD, Racapé J, Gervy C, Husson C, Dhaene M, Wissing KM, Nortier JL. Biochemical parameters after cholecalciferol repletion in hemodialysis: results From the VitaDial randomized trial. *Am J Kidney Dis* 2014; **64**: 696-705 [PMID: 24856872 DOI: 10.1053/j.ajkd.2014.04.020]

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## Hyporeninemic hypoaldosteronism and diabetes mellitus: Pathophysiology assumptions, clinical aspects and implications for management

André Gustavo P Sousa, João Victor de Sousa Cabral, William Batah El-Feghaly, Luísa Silva de Sousa, Adriana Bezerra Nunes

André Gustavo P Sousa, Adriana Bezerra Nunes, Department of Clinical Medicine, Federal University of Rio Grande do Norte, Natal, RN 59012-300, Brazil

João Victor de Sousa Cabral, William Batah El-Feghaly, Luísa Silva de Sousa, Onofre Lopes University Hospital, Federal University of Rio Grande do Norte, Natal, RN 59012-300, Brazil

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**Correspondence to:** André Gustavo P Sousa, MD, PhD, Associate Professor, Department of Clinical Medicine, Federal University of Rio Grande do Norte, Av Nilo Peçanha, 620, Petrópolis, Natal, RN 59012-300, Brazil. [agpsousa@ig.com.br](mailto:agpsousa@ig.com.br)  
Telephone: +55-84-33429706  
Fax: +55-84-33429706

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### Abstract

Patients with diabetes mellitus (DM) frequently develop electrolyte disorders, including hyperkalemia. The most important causal factor of chronic hyperkalemia in patients with diabetes is the syndrome of hyporeninemic hypoaldosteronism (HH), but other conditions may also contribute. Moreover, as hyperkalemia is related to the blockage of the renin-angiotensin-aldosterone system (RAAS) and HH is most common among patients with mild to moderate renal insufficiency due to diabetic nephropathy (DN), the proper evaluation and management of these patients is quite complex. Despite its obvious relationship with diabetic nephropathy, HH is also related to other microvascular complications, such as DN, particularly the autonomic type. To confirm the diagnosis, plasma aldosterone concentration and the levels of renin and cortisol are measured when the RAAS is activated. In addition, synthetic mineralocorticoid and/or diuretics are used for the treatment of this syndrome. However, few studies on the implications of HH in the treatment of patients with DM have been conducted in recent years, and therefore little, if any, progress has been made. This comprehensive review highlights the findings regarding the epidemiology, diagnosis, and management recommendations for HH in patients with DM to clarify the diagnosis of this clinical condition, which is often neglected, and to assist in the improvement of patient care.

**Key words:** Hyporeninemic; Diabetes; Hyperkalemia; Renal tubular acidosis; Hypoaldosteronism

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**Core tip:** Hyporeninemic hypoaldosteronism is the most significant cause of hyperkalemia in patients with diabetes mellitus. In order to help physicians, diabetologists, and endocrinologists in proper management of this condition, this review will focus on the current available evidence, highlighting the consequences of this condition for the treatment of arterial hypertension and proteinuria in these patients.

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## INTRODUCTION

Hyperkalemia, usually defined by a potassium concentration greater than 5.5 or 6.0 mEq/L (mmol/L)<sup>[1]</sup>, is a common clinical condition and is potentially life-threatening due to the risk of ileus paralysis and fatal arrhythmias<sup>[2]</sup>. Among the various causes of hyperkalemia, hypoaldosteronism should be considered in any patient with persistent hyperkalemia for which there is no clear cause, such as renal failure, the use of potassium supplements or a potassium-sparing diuretic. The causes of hypoaldosteronism include both acquired (secondary mineralocorticoid deficiency) and, less often, inherited disorders (primary mineralocorticoid deficiency), which can affect adrenal aldosterone synthesis or renal (and maybe adrenal) renin release. The most common secondary mineralocorticoid deficiency cause is hyporeninemic hypoaldosteronism (HH), including cases related to diabetes mellitus (DM), pharmacologic inhibition of angiotensin II, the use of potassium-sparing diuretics, and non-steroidal anti-inflammatory drugs (NSAIDs) or calcineurin inhibitors<sup>[3]</sup>. Primary hypoaldosteronism can be the result of acquired or congenital errors in renal juxtaglomerular function, angiotensin generation or activity, or aldosterone synthesis. Secondary hypoaldosteronism (pseudohypoaldosteronism), in contrast, occurs as a consequence of mutations in genes that may adversely affect aldosterone-mediated electrolyte homeostasis<sup>[4]</sup>.

For decades, it has been known that there is a relationship between the metabolism of glucose and potassium; the lack of insulin predisposes one to hyperkalemia, exogenous insulin lowers serum potassium, and potassium deficiency interferes with insulin release, leading to glucose intolerance<sup>[5]</sup>. In addition, hyperkalemia occurs more frequently in patients with DM than in the general population<sup>[6]</sup>. Various mechanisms are involved in the development of hyperkalemia in patients with DM, for example hyperosmolality, insulin deficiency or resistance, HH, potassium-sparing drugs, and raised

glucagon concentrations<sup>[2]</sup>. HH is related to a secondary mineralocorticoid deficiency, leads to hyperkalemia accompanied by urinary salt wasting<sup>[7]</sup> and is commonly seen in association with diabetic nephropathy (DN). HH normally occurs when there is some underlying renal pathology causing volume expansion<sup>[8]</sup>.

A few years ago, a series of publications addressed the close relationship between HH and DM in case reports and studies on physiology and applied pathophysiology. However, most of these studies were published before the spread of the use of medications that interfere with the renin-angiotensin-aldosterone system (RAAS), such as inhibitors of the angiotensinogen-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs). Currently, few studies have reported on the implications of the diagnosis of HH in patients with DM, the concerns of a diagnosis of HH for the management of antihypertensive medications (ACEIs and ARBs) and the natural history of nephropathy in patients with DM. The objective of this review is to highlight the pathophysiology and diagnosis of HH in patients with DM, as well as the consequences of this condition for the treatment of arterial hypertension (AH) and proteinuria in these patients.

## PATHOPHYSIOLOGY

For the purpose of maintaining homeostasis, the urinary excretion of potassium is typically equal to the quantity ingested minus the quantity excreted in the feces<sup>[6]</sup>. In normal individuals, most of the potassium filtered at the glomerulus is reabsorbed in the proximal tubule and in the ascending limb of Henle's loop, and most of the potassium excreted in the urine is that secreted by the distal convoluted tubule and the cortical collecting tubule (CCT)<sup>[7]</sup>. Consequently, potassium secretion in the cortical collecting duct is the major determinant of urinary potassium excretion<sup>[6]</sup>. However, the amount of potassium finally excreted in the urine is typically less than the amount secreted by earlier segments because there is a considerable quantity of potassium reabsorbed at the outer medullary collecting duct<sup>[3]</sup>. Potassium enters the tubular cell in exchange for sodium by the action of the Na-K ATPase located at the basolateral membrane, an active transport mechanism that moves three sodium ions out of the cell while simultaneously carrying two potassium ions into the cell. This process is able to maintain a high potassium and a low sodium concentration in the cell. Aldosterone stimulates Na-K ATPase activity directly and increases luminal membrane permeability to sodium. It also increases the permeability of the luminal membrane to potassium<sup>[6]</sup>. Aldosterone thus plays a major role in regulating the renal excretion of potassium. The action of aldosterone is to increase the number of open sodium channels in the luminal membrane of the principal cells in the CCT, leading to increased sodium reabsorption. The subsequent elimination of sodium from the tubular

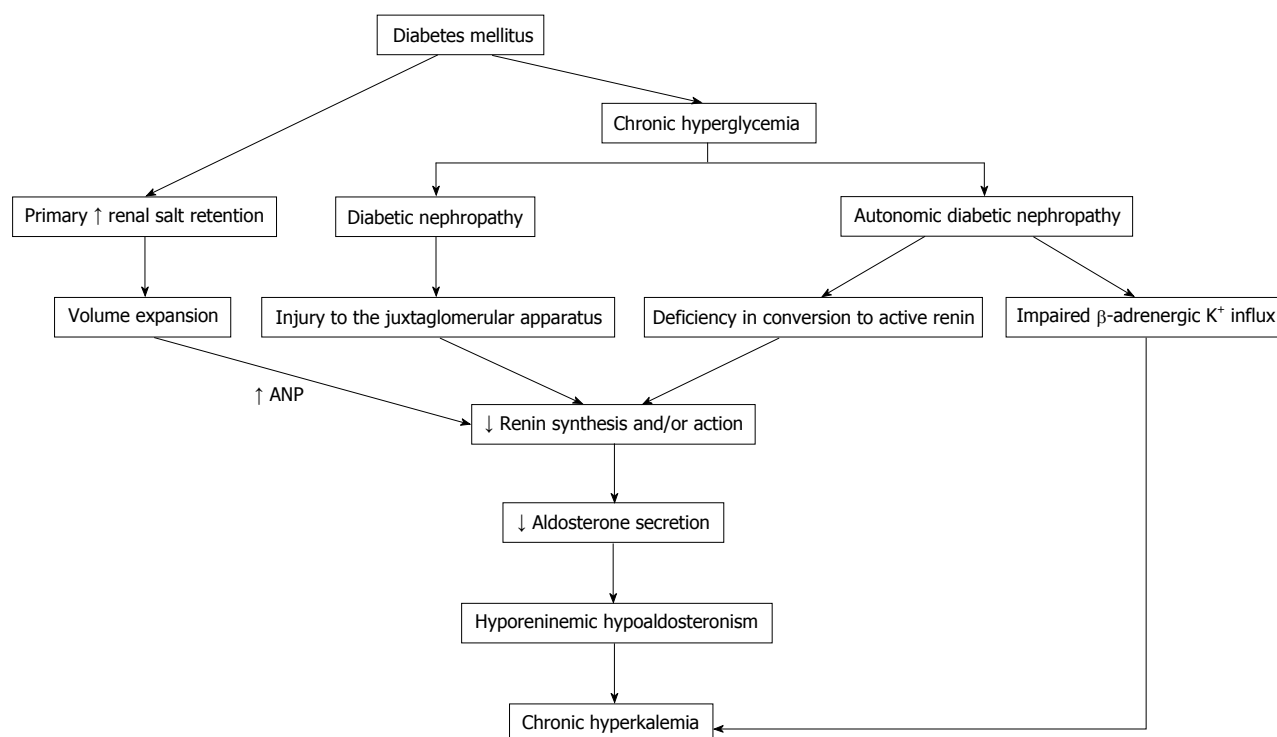


Figure 1 Pathophysiology of hyporeninemic hypoaldosteronism related to diabetes mellitus. ANP: Atrial natriuretic peptide; K<sup>+</sup>: Potassium.

fluid makes the lumen electronegative, thereby creating an electrical gradient that stimulates the secretion of potassium into the lumen through potassium channels in the luminal membrane<sup>[7]</sup>.

HH is a syndrome that is related to a reduction in the production of aldosterone from the adrenal gland and in the release of renin from the juxtaglomerular cells. A reduced renin release results in a decreased systemic and intra-adrenal angiotensin II (Ang II) production, which contributes to the decline in aldosterone secretion<sup>[9]</sup>. Ang II is a cofactor, along with potassium, in aldosterone synthesis by the adrenal gland. The production of renin occurs in the kidney by the juxtaglomerular cells and is stimulated primarily by the reduction in intravascular volume. Therefore, the RAAS plays a key role in blood pressure regulation<sup>[7]</sup>. HH is most common among patients with mild to moderate renal insufficiency due to DN or chronic interstitial nephritis<sup>[10]</sup>. As seen in Figure 1, the reduction in renin release in patients with DM may be secondary to<sup>[10]</sup>: (1) injury to the juxtaglomerular apparatus (such as an afferent arteriolar hyalinization); (2) defects in the stimulation factors or suppressed plasma renin activity (PRA) because of the deficiency in conversion of prorenin (big renin) to active renin; (3) autonomic dysfunction (as part of the autonomic diabetic neuropathy); or (4) an primary increase in renal salt retention with volume expansion, which suppresses renin synthesis (paradoxically, if compared to individuals without DM). The volume expansion leads to the suppression of renin release because of an increase in release of atrial natriuretic peptide, thus contributing to HH<sup>[11]</sup>. It is important to emphasize that any condition in which

the renin-angiotensin-aldosterone axis is interrupted can potentially produce type IV renal tubular acidosis (RTA); therefore, type IV RTA and HH are often considered synonymous. Renal tubular damage may cause inadequate renin production and release, adrenal dysfunction may lead to inadequate aldosterone production, and the principal cells of the CCT may not respond normally to aldosterone. In addition, atrophy of the juxtaglomerular apparatus may be present, and this may be more prevalent among diabetic patients. This atrophy is related to autonomic neuropathy<sup>[12]</sup>, chronic hyperkalemia and volume expansion<sup>[11]</sup>. In addition, any combination of these factors may cause HH or type IV RTA. Indeed, all of these factors (or any combination of them) may be present in some patients<sup>[13]</sup>.

Because there is a reduced secretion of potassium, which can lead to chronic hyperkalemia, the resulting hyperkalemia impairs NH<sub>4</sub><sup>+</sup> production in the collecting duct. This leads to impaired generation of acid for excretion and metabolic acidosis<sup>[14]</sup>, usually hyperchloremic metabolic acidosis, due to the deficiency in potassium and hydrogen secretion<sup>[15]</sup>. The degree of acidosis varies and may be related to the underlying chronic kidney disease (CKD). In type I (*i.e.*, distal) RTA, the defect is in proton secretion with a resulting high urine pH (> 5.3), whereas in type IV RTA, the primary defect is in ammoniagenesis. This defect, albeit significant, still permits elaboration of acidic (pH < 5.3) urine. Hyperkalemia inhibits renal ammoniagenesis in several ways that involve the direct effects of one on the other: Modulation of ion transport by aldosterone, lowering of ammonia formation, and defective medullary ammonium handling. Furthermore, it may produce

acidosis by shifting protons from inside the cells to the extracellular space as homeostatic mechanisms attempt to buffer potassium by intracellular uptake<sup>[2]</sup>.

## EPIDEMIOLOGY

HH predominantly occurs in patients 50 to 70 years of age with DN and/or chronic tubulointerstitial disease who have mild to moderate kidney failure<sup>[16]</sup>. It is more frequent among women. Therefore, patients with these characteristics should be monitored<sup>[17]</sup>. Other common clinical conditions associated with HH include various forms of interstitial disease, such as amyloid, monoclonal gammopathies and the interstitial nephritis associated with NSAIDs in particular<sup>[2,18-20]</sup>. In addition, there is a close relationship between diabetes and hyperkalemia. Insulin deficiency, kidney disease, HH, and use of medications such as ACEIs and ARBs that increase the risk of hyperkalemia are mechanisms involved in this relationship<sup>[21]</sup>. Therefore, hyperkalemia can be observed in type 1 DM patients due to their insulin deficiency and ketone-prone condition, as well as in type 2 DM patients, in whom an association has been found between serum potassium concentration and incidence of hyperkalemia and insulin resistance (estimated by the homeostasis model assessment)<sup>[21]</sup>. In addition, serum potassium concentration is likely to be more increased in patients with poorly controlled type 2 DM with insulin resistance than in those without DM<sup>[22]</sup>. Clinicians must also be aware that hyperkalemia in patients with type 1 DM may be due to concurrent adrenal insufficiency in the case of autoimmune polyglandular syndrome<sup>[23]</sup>. Specifically about HH, in many cases, particularly DN, hypoaldosteronism and low renin levels are present. In previous studies, about half of the subjects with HH were found to be diabetics<sup>[10]</sup>. Moreover, although HH usually occurs with persistent high potassium levels, some cases of HH are not accompanied by hyperkalemia despite suppression of renin and aldosterone levels. A previous study assessed the renin-aldosterone axis in 13 normokalemic patients with DM and creatinine clearances of < 40 mL/min and showed that approximately 92.3% of them had HH<sup>[24]</sup>. However, no recent studies have assessed the prevalence of HH in individuals with DM, particularly among those with normal renal function and/or without hyperkalemia. Similarly, the incidence of hyperkalemia in patients with HH could not be found. However, such studies are difficult to conduct because HH is often underdiagnosed and habitually only manifests when the patient is challenged by excess dietary potassium or by exposure to medications; furthermore, HH improves upon the removal of the exacerbating agents. In addition, it is worth noting that these older studies were conducted when the use of ACEIs and ARBs was not widespread. Recently, only a few studies have been conducted with moot methodology to determine the current incidence and prevalence of HH among populations that include patients using these medications. One recent study<sup>[25]</sup>

evaluated the prevalence and role of type IV RTA in the development of significant hyperkalemia in patients admitted to a hospital for over 1 year and found significant hyperkalemia (> 6.0 mEq/L) in 3.8% of hospital admissions. Type IV RTA was diagnosed in 42% of these patients, of whom 71% had pre-existing renal insufficiency due to DN or tubulointerstitial nephritis. In the same study, patients with type IV RTA more frequently had a history of DM (50% vs 24%,  $P = 0.07$ ) and were more likely to have pre-existing kidney failure (71% vs 38%,  $P < 0.05$ ). No significant difference in the use of ACEIs, ARBs, NSAIDs, or another potassium-sparing diuretics was found between the groups with or without type IV RTA<sup>[25]</sup>. In another pharmacovigilance study, life-threatening hyperkalemia was found to be related to polypharmacy (use of more than 5 drugs), age (greater than 74 years), gender (female) and glomerular filtration rate (GFR) < 60 mL/min<sup>[26]</sup>.

## CLINICAL FEATURES AND DIAGNOSIS

In patients with persistent hyperkalemia with no obvious cause (such as renal failure, the use of potassium supplements or a potassium-sparing diuretic), the diagnosis of hypoaldosteronism should be considered<sup>[9]</sup>. These patients typically have elevated potassium serum levels disproportional to their renal function and potassium intake. Actually, HH accounts for most cases of unexplained hyperkalemia in patients in whom GFR and potassium intake would not be expected to result in hyperkalemia<sup>[2]</sup>. Regarding its clinical manifestation, most patients with HH are asymptomatic and have mild to moderate hyperkalemia. However, patients with HH may have significant hyperkalemia with no manifestations for long periods of time and may occasionally be identified in routine laboratory tests<sup>[6]</sup>. Conversely, an acute event (renal dysfunction or salt restriction) or medication (such as ACEIs, ARBs, potassium-sparing diuretics or heparin) may sometimes precipitate hyperkalemia in a patient whose disease has not been recognized because his or her plasma potassium has not exceeded the normal range. In those cases, acute hyperkalemia can disturb excitable tissues and provide different manifestations depending on the potassium serum level. Nausea, muscle weakness, paresthesias and fasciculations may occur and could progress to paralysis in severe cases. The progressive effects on the heart can be seen in the electrocardiogram (ECG), namely the peaking of T waves, ST-segment depression, widening of the PR interval, widening of the QRS interval, loss of the P wave, and development of a sine-wave pattern<sup>[3]</sup>, and may even culminate in ventricular fibrillation. Generally, with acute onset of hyperkalemia, ECG changes appear at a serum potassium level of 6-7 mEq/L (6-7 mmol/L). However, with chronic hyperkalemia, the ECG may remain normal up to a concentration of 8-9 mEq/L (8-9 mmol/L)<sup>[3]</sup>. Despite these findings, a retrospective study revealed a poor correlation between serum potassium concentrations and cardiac manifestations<sup>[27]</sup>.

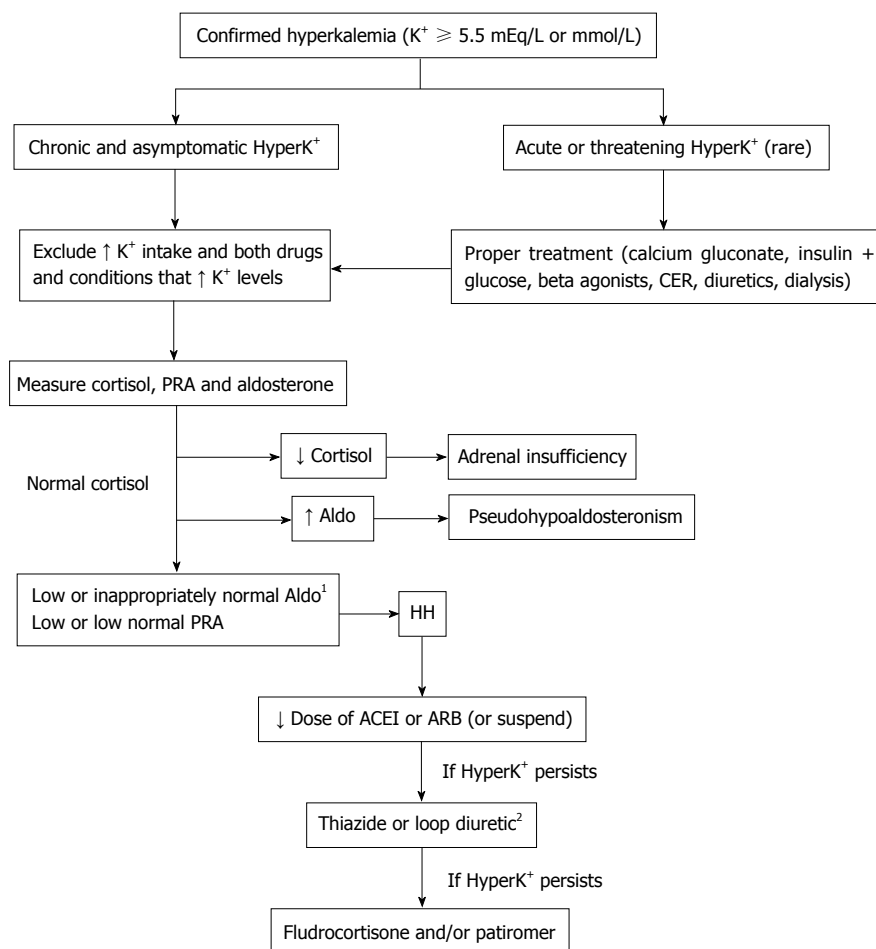
Furthermore, hypoaldosteronism has been related with mild hyperchloremic metabolic acidosis. Metabolic acidosis is primarily the result of impaired renal ammoniogenesis caused by hyperkalemia (type IV RTA), reduced aldosterone levels, and reduced distal delivery of sodium. The acidosis is hyperchloremic because the renal insufficiency is mild and the retention of uremic anions is slight. Patients' urinary pH is characteristically acidic because impaired ammoniogenesis reduces the buffering capacity of urine; occasionally, patients cannot acidify urine because of an associated distal tubular defect in hydrogen ion secretion<sup>[6]</sup>. Chronic hyperkalemia, *per se*, is usually asymptomatic, but chronic acidosis contributes to long-term morbid conditions, including bone demineralization<sup>[28]</sup>. Hyponatremia is uncommon in HH because there is no hypovolemia-induced stimulation to release antidiuretic hormone (ADH) and the plasma cortisol level, a tonic inhibitor of ADH release, is normal. Otherwise, when hyponatremia is present, other disorders such as primary adrenal insufficiency should be suspected<sup>[9]</sup>.

Regarding the diagnosis of HH, it is important to remember that middle-aged or elderly patients with chronic hyperkalemia, diabetes, and/or with renal insufficiency are at risk. However, patients with DM at any age and metabolic conditions can be at risk. A study performed some years ago described a 31-year-old man with insulin-dependent DM and previous normal renal function who presented with symptomatic hyperkalemia and reversible impairment of renal function when treated with enalapril<sup>[29]</sup>. Before performing laboratory and dynamic tests to confirm HH, patients should be questioned about increased dietary potassium intake (including fruit juices and herbal preparations, such as noni), cell lysis (rhabdomyolysis), and the use of medications that interfere with potassium levels, such as ACEIs, ARBs, NSAIDs, beta-blockers, calcineurin inhibitor (cyclosporine, for example) and heparin, as well as human immunodeficiency virus (HIV) infection<sup>[6]</sup>. Patients with HIV are at risk for adrenal insufficiency, which may present as hyperkalemia. Nevertheless, the adrenal defect is sometimes selective for mineralocorticoid production. Furthermore, trimethoprim, a drug commonly used in chemoprophylaxis regimens for patients with AIDS, may impair tubular potassium excretion and may cause hyperkalemia<sup>[3]</sup>. In women, the use of some oral contraceptives should be evaluated because the progestin drospirenone retains mineralocorticoid blocking effects similar to those seen with spironolactone<sup>[30]</sup>. Other rarer diseases that can also cause nephropathy and HH may need to be excluded, for example, multiple myeloma, amyloidosis, systemic lupus erythematosus, and genetic disorders (pseudohypoaldosteronism)<sup>[7]</sup>. Some researchers also recommend excluding alcohol consumption, hemolysis, rhabdomyolysis, and/or metabolic acidosis<sup>[2]</sup>. In addition, as revealed before, clinicians must also be aware that hyperkalemia in patients with type 1 DM may be due to concurrent adrenal insufficiency in the case of

autoimmune polyglandular syndrome.

As previously mentioned, HH should be suspected in cases of unexplained chronic hyperkalemia in patients in whom GFR and potassium intake would likely not result in hyperkalemia. After exclusion of those possible causes, to confirm and to perform a differential diagnosis of hypoaldosteronism, it is recommended that the PRA, serum aldosterone, and serum cortisol should be measured (Figure 2). Some authors<sup>[9]</sup> recommend that these tests should be performed after the administration of a loop diuretic (furosemide) or after three hours in the upright position, which increases renin and aldosterone release in normal individuals. This ensures activation of the RAAS and the reliability of the findings, especially in the HH context. Hyperkalemia in patients with chronic kidney failure that is well short of end-stage (stage V) is typically characterized by plasma aldosterone levels that are inappropriately low for the degree of hyperkalemia<sup>[13]</sup>. Therefore, the authors of this article believe that, after excluding other causes, the existence of significant hyperkalemia (potassium levels > 5.5–6.0 mEq/L) accompanied by low or inappropriately normal aldosterone levels is indicative of hypoaldosteronism. In addition, HH is characterized by a low (or low normal) PRA and commonly normal serum cortisol<sup>[9]</sup>. Conversely, in the absence of significant hyperkalemia, it is important to ensure activation of the RAAS by the furosemide or upright test. These tests have commonly been used to differentiate between aldosterone-producing adenoma and idiopathic hyperaldosteronism in cases of primary aldosteronism<sup>[31]</sup>, but they have recently been used increasingly less often for this purpose<sup>[32]</sup>. In brief, after a minimum of 1 wk on a 90 mmol/d sodium diet and in a potassium-replete state, the furosemide test can be performed after administration of a dose of 2 mg/kg of body weight orally at 06:00. The dose is then repeated 6 h later at 12:00 (if systolic blood pressure is  $\geq$  120 mmHg without orthostatic hypotension). At 1800 (6 h after the second dose of furosemide), a blood sample to measure PRA and aldosterone, sodium, and potassium concentrations is obtained after 5 min in the sitting position<sup>[31]</sup>. Other authors have used a combined test (upright position with administration of 60 mg furosemide)<sup>[33]</sup> for clinical research purposes in particular. In cases of primary adrenal insufficiency, both serum aldosterone and cortisol concentrations are typically low and PRA high. Ultimately, unlike primary hyperaldosteronism, there are no cutoffs for the diagnosis of HH. Consequently, reference values of laboratory tests are commonly used. Considering the lack of recent data concerning the diagnosis of HH, the impact of ACEI and ARB use is unknown. These medications are known to alter the aldosterone:PRA ratio by increasing the PRA<sup>[34]</sup>, potentially reducing aldosterone levels. Therefore, there may be doubt regarding whether these medications interfere with the diagnosis of HH. In the absence of specific studies, the authors of this article believe that the suspension of ACEIs and ARBs is not essential to establish a diagnosis of HH. If a patient using these





**Figure 2** Diagnosis and management of hyporeninemic hypoaldosteronism related to diabetes mellitus. <sup>1</sup>In any cases, it might be necessary to activate the renin-angiotensin-aldosterone system (using a loop diuretic or the upright position); <sup>2</sup>After using diuretics, the use of ACEIs or ARBs should be restarted (if suspended). ACEIs: Angiotensinogen-converting enzyme inhibitors; Aldo: Aldosterone; ARBs: Angiotensin receptor blockers; CER: Calcium exchange resins; HyperK<sup>+</sup>: Hyperkalemia; K<sup>+</sup>: Potassium; PRA: Plasma renin activity; HH: Hyporeninemic hypoaldosteronism.

drugs develops hyperkalemia without impaired renal function and has low or inappropriately normal aldosterone and PRA levels, the most likely diagnosis is HH. However, if the suspension of drugs is necessary (either because of continuing concerns about the diagnosis or because of hyperkalemia), aldosterone and PRA should be repeated. Potassium-sparing diuretics (such as spironolactone and eplerenone), however, must be suspended before hormonal laboratory evaluation. Finally, further studies are clearly needed to clarify these issues.

## RELATION TO OTHER DIABETIC MICROVASCULAR COMPLICATIONS

As previously stated, HH is most often found in patients with DN. It is therefore more likely to diagnose HH in patients with other diabetic microvascular complications. Considering the limited number of recent studies, few (if any) advances in the understanding of this issue have been obtained. Obviously, a key point in the explanation of these findings is hyporeninemia. Its association with DN is at least in part related to the damage to the juxtaglomerular apparatus and problems in the conversion

of prorenin to active renin in diabetic patients<sup>[16]</sup>. The most comprehensive hypothesis accounting for the hyporeninemia is the associated renal disease-associated destruction of renin-producing cells (JG cells) or impaired function of the juxtaglomerular apparatus. However, the pathological findings of the juxtaglomerular apparatus in HH are limited in number and are likely nonspecific (they are found in essential hypertension, for instance)<sup>[10]</sup>. In addition, previous studies have suggested that impaired conversion of prorenin to active renin may cause a clinically significant reduction of PRA<sup>[35]</sup>. Nevertheless, the molecular mechanisms that explain how renal dysfunctions affect this conversion remain uncertain. A study designed to clarify the cause of selective HH divided 118 normokalemic patients based on presence or absence of diabetic neuropathy and/or nephropathy, and the authors observed that the development of abnormalities was associated with diabetic neuropathy and/or nephropathy<sup>[33]</sup>. According to a different study<sup>[36]</sup>, autonomic neuropathy may be more relevant in HH than nephropathy: In patients with type 2 DM and autonomic neuropathy, levels of PRA were lower and inactive renin levels were higher, regardless of proteinuria

and GFR. In addition, a Japanese study on the postural test and neuropathic diabetes showed that autonomic dysfunction was a major factor in impairing the processing of prorenin to active renin in diabetic patients and that severe autonomic dysfunction may impair the biosynthesis of prorenin in patients with HH<sup>[37]</sup>. In fact, autonomic neuropathy is known to result in impaired beta2-mediated influx of potassium into cells<sup>[38]</sup>. In addition, the stimulation of beta-adrenergic receptors induces renin release<sup>[39]</sup>; autonomic neuropathy with consequent sympathetic insufficiency might therefore lead to an impairment of renin production in patients with DM<sup>[10]</sup>. Theoretically, the suppression of PRA that results from sympathetic dysfunction should lead to a reduction in blood pressure; however, most reported patients with HH have AH<sup>[10]</sup>. Thus, these data reveal a significant association between other diabetic microvascular complications and HH, but no single pathophysiological aspect is able to accurately explain the findings observed related to DM-HH. It is most likely that there is a combination of mechanisms that together explain HH within the spectrum of microvascular diabetic complications.

## IMPLICATIONS FOR THE MANAGEMENT OF AH AND PROTEINURIA

Patients with DM commonly use ACEIs and ARBs when they have concurrent hypertension and/or DN or for cardiovascular protection<sup>[40]</sup>. Despite the decrease in PRA in DN<sup>[34]</sup>, the RAAS is fundamental in DN pathogenesis; there is an increased production of Ang II secondary to stimulation by hyperglycemia and advanced glycation end products. Ang II promotes an increase in intraglomerular pressure and adrenal aldosterone production. In addition, prorenin and aldosterone contribute to renal fibrosis<sup>[34]</sup>. Therefore, ACEIs and ARBs are essential for nephroprotection. However, one of the main adverse effects of these medications is hyperkalemia. The patients at the highest risk for hyperkalemia include those with either diabetes or those with impaired renal function in whom a defect in the excretion of renal potassium may already exist<sup>[41]</sup>, including patients with HH. The development of hyperkalemia as a direct or indirect consequence of decreased aldosterone concentrations is typically observed when aldosterone concentrations have already decreased prior to the administration of drugs<sup>[41]</sup>. DN is the most common cause of HH, ranging from 43% to 63% of cases<sup>[9,42]</sup>. In addition, the risk of hyperkalemia increases with the progression of DM as a result of insulin deficiency, by limiting the body's ability to shift potassium into cells<sup>[41]</sup>. ACEIs and ARBs impair the urinary excretion of potassium by inhibiting the stimulatory effect of Ang II on aldosterone secretion in the adrenal gland. ACEIs act by blocking the formation of Ang II, whereas ARBs prevent Ang II from binding to its adrenal receptor, both systemically and perhaps within the adrenal zona glomerulosa<sup>[43]</sup>. However, while the effects of these

medications in patients with HH can be inferred, they have not been evaluated through specific clinical studies.

The role of RAAS inhibition in postponing the progression of DN by using ACEIs or ARBs has been well established in multiple controlled trials; these medications are thus the drugs of choice in the treatment of hypertension and/or proteinuria in patients with DM<sup>[41]</sup>. In adult patients with diabetes and kidney disease, ACEIs and ARBs have been the most effective strategies in preventing progression to end-stage kidney disease<sup>[44]</sup>. Unfortunately, the true prevalence of HH in patients with DM, especially those with DN, is not known. However, given the vast prevalence of DM worldwide, it is thought that there are many patients with HH (even with normal levels of potassium) using ACEIs or ARBs. The large clinical trials that evaluated the efficacy of ACEIs, ARBs, and direct renin inhibitors on the natural history of DN did not perform post hoc analyses regarding the status of the RAAS (baseline levels of aldosterone and PRA, for example). HH has been suggested to be nephroprotective on its own, as it leads to lower levels of aldosterone and PRA<sup>[45]</sup>. Thus, one might suggest that the use of medications that interfere with the RAAS would have a modest to no effect on the progression of DN and would only increase the risk of hyperkalemia. However, these issues have not been specifically evaluated and are therefore only suppositions. In the absence of contrary evidence, the authors still believe that ACEIs and ARBs can be used in patients with HH and may even have clinical benefits, since their potassium levels do not present an obstacle and are closely monitored. In fact, the systematic monitoring of potassium levels in patients using RAAS blockers prevents cases of severe hyperkalemia. A study found that patients who received potassium monitoring were 50% less likely to experience a hyperkalemia-associated adverse event [adjusted relative risk of 0.50 (95%CI: 0.37-0.66)] compared to patients without monitoring. In patients with CKD, the adjusted relative risk was even lower at 0.29 (95%CI: 0.18-0.46)<sup>[46]</sup>.

Given the underlying pathophysiology, there was hope that dual RAAS blockade could reduce the progression of DN even further<sup>[34]</sup>. The potential benefit of this dual blockade has been tested in three large randomized clinical trials, and unfortunately, their results demonstrated a lack of benefit with regard to renal or cardiovascular outcomes in diabetic patients<sup>[47-49]</sup>. In contrast, a recent meta-analysis found that the progression to end-stage renal disease was significantly less likely after combined treatment with an ARB and an ACEI<sup>[44]</sup>. However, one of the main safety concerns with more intensive RAAS blockade is hyperkalemia. Thus, careful monitoring of potassium in CKD patients in whom a mineralocorticoid receptor blocker is to be used in combination with an ACEI or ARB is of utmost importance, especially if the patient also has diabetes. While precaution may suggest that this combination should be avoided, it is widely used, especially in cases

of heart failure. Prior studies have shown that the risk factors for hyperkalemia with the use of RAAS blockers include older age, lower GFR, higher baseline potassium levels, and the use of more than one medication that interferes with potassium excretion<sup>[50]</sup>. In fact, dual RAAS blockade has been shown to increase the risk of hyperkalemia and acute kidney injury. New agents for the treatment of hyperkalemia may increase the feasibility of dual blockade of RAAS; however, further research is still needed<sup>[34]</sup>. Nevertheless, withholding or withdrawing drugs that block the RAAS on the basis of impaired kidney function or on diagnosis of HH alone may potentially deprive many patients of the cardiovascular benefit they would receive; instead, there are numerous steps that can be taken to minimize the risk of hyperkalemia<sup>[3,41]</sup>. The initial approach should be to estimate the GFR and the potassium levels to assess the specific risk of hyperkalemia as well as to review the patient's medications profile. Drugs that can impair renal potassium excretion<sup>[41,51]</sup> should be discontinued. It is important to inquire specifically about the use of over-the-counter NSAIDs and herbal remedies, as herbs can be a hidden source of intake potassium. A low-potassium diet should then be prescribed with specific counseling against the use of potassium-containing salt substitutes. Next, therapy with a low dose of ACEIs or ARBs should be initiated. It is essential to monitor patients' potassium levels within 1 wk after initiating therapy or after increasing dosage<sup>[52]</sup>. If potassium levels remain normal, the dose of the drug can be titrated upwards. If potassium is higher than 5.5 mEq/L (5.5 mmol/L) despite the steps described above, ACEIs and ARBs may need to be avoided<sup>[41,51,52]</sup>. When albuminuria and proteinuria occur in DN, RAAS inhibitors should be used aggressively. However, the antiproteinuric effects of ACEIs and ARBs can be observed soon after starting treatment and may decrease the effectiveness throughout the treatment<sup>[40,53]</sup>. In addition, when an ARB is combined with an ACEI or direct renin inhibitor (such as aliskiren), there has been no evidence of clinical efficacy and adverse reactions have in fact been increased<sup>[40]</sup>. Despite the fact that ACEIs and ARBs are key drugs for the treatment of DM nephropathy, new treatment strategies are needed to achieve improved effectiveness. Finally, studies evaluating the efficacy and safety of medications in the treatment of hypertension and albuminuria in patients with HH are highly needed.

## MANAGEMENT OF HYPERKALEMIA IN PATIENTS WITH HH

The incidence of hyperkalemia is higher in diabetic patients than in the general population<sup>[52]</sup>, and the most common causal factor of chronic hyperkalemia in patients with diabetes is the reduced tubular secretion of potassium due to HH. The development of overt hyperkalemia is most common in patients with other risk factors that further impair the efficiency of potassium

excretion, such as renal insufficiency, volume depletion, or the use of medications that interfere through the deterioration of intravascular volume contraction<sup>[2]</sup>. Indeed, worsening of renal function and hyperkalemia may occur in patients who are using the novel sodium glucose cotransporter 2 inhibitors, particularly those predisposed to hyperkalemia due to impaired renal function, medications, or other medical conditions<sup>[54]</sup>.

There are no recognized recommendations or guidelines regarding when to initiate hyperkalemia treatment, but it is usually necessary to treat hyperkalemia because of the potential clinical manifestations in excitable tissue and the risk of progression to respiratory failure and fatal arrhythmias<sup>[3]</sup>. A summary of the management of hyperkalemia in patients with DM is presented in Figure 2. In cases of rapid elevation, very high potassium levels, and in life-threatening conditions, emergency treatment should be promptly initiated. Hyperkalemia must be acutely treated to counter its cardiac effects, using calcium gluconate or chloride to decrease the membrane excitability of the cardiac cells and reverse the locking depolarization caused by hyperkalemia. Drugs that provide potassium redistribution between the intra and extracellular fluid should be used. Insulin shifts the potassium into the cell, and the recommended dose is 10 units of regular insulin intravenously together with 50 mL of 50% glucose to prevent hypoglycemia in patients with glycemia below 200-250 mg/dL. Beta agonists, such as salbutamol, are also used to redistribute the potassium and to act synergistically with insulin. The recommended dose is 10-20 mg of nebulized salbutamol in 4 mL of saline solution for 10 min. For potassium removal, calcium exchange resins, diuretics and/or dialysis can be used. The use of bicarbonate infusion should be restricted to patients with associated metabolic acidosis and when it is non-gap metabolic acidosis<sup>[27,30]</sup>. In addition, the most widely used calcium exchange resin is sodium polystyrene sulfonate. As its effect is slow and there is a potential risk of intestinal injury, it is recommended that the use of this medication be restricted to the acute management of hyperkalemia only and when dialysis is not available or indicated<sup>[32]</sup>; it must not be used chronically.

In patients with HH in particular, the use of medications that affect the RAAS should be reassessed. In cases of hyperkalemia (serum potassium concentration up to 5.5 mEq/L or mmol/L) in patients with HH using ACEIs, dose reduction may be initially attempted<sup>[3]</sup>. In some cases, potassium concentration will improve, allowing the patient to remain on the renin-angiotensin blocker, although at a lower dose. ARBs and direct renin inhibitors should be used with the same caution. It is important to remember to recommend a low potassium diet and to avoid the use of NSAIDs, including selective cyclooxygenase-2 inhibitors<sup>[41]</sup>. Some years ago, a case of selective HH triggered by the use of NSAID was reported<sup>[55]</sup>. Dual RAAS blockade must be avoided (combinations of ACEIs and ARBs or direct renin inhibitors).

However, the use of aldosterone antagonists (spironolactone or eplerenone) is relatively common. If indicated, the dose of spironolactone should not exceed 25 mg daily when used with an ACEI or ARB, and this combination should be avoided when the GFR is < 30 mL/min. Patients with HH and decompensated diabetes (with significant hyperglycemia, particularly if there is concomitant loss of weight) have an additional increased risk of hyperkalemia; insulin deficiency contributes to both a low serum aldosterone concentration and to an increased concentration of extracellular potassium. In addition to the risk associated with insulin deficiency, hyperglycemia creates a differential osmolality, resulting in a hypertonic extracellular fluid. Consequently, water and ions, as potassium, are attracted to the extracellular fluid. In these patients, the use of insulin for the treatment of hyperglycemia may be recommended<sup>[3]</sup>. If these actions do not have the expected result and the risk benefit profile is in favor of maintaining the RAAS blockade, the use of fludrocortisone or diuretics can be attempted. Fludrocortisone is a potent mineralocorticoid that promotes increased reabsorption of sodium and loss of potassium from renal distal tubules<sup>[9]</sup>. While being administered, it is necessary to monitor the concentration of serum sodium and potassium because of the potential risk of hypokalemia and hypokalemic alkalosis<sup>[20,31,52]</sup>. The typical dose of fludrocortisone required to normalize the serum potassium is usually higher than the dose in primary adrenal insufficiency (0.05 to 0.2 mg daily). Nevertheless, the authors recommend starting with 0.1 to 0.2 mg once a day and increasing the dosage based on potassium levels and signs of hypervolemia. However, fludrocortisone is not widely used; patients with HH often have hypertension and/or edema (heart failure, for instance) and thus should either use fludrocortisone with caution or not use it at all because of its effect on plasma volume expansion. In those cases, hyperkalemia can be treated with low potassium ingestion and drugs that eliminate potassium, such as diuretics<sup>[33,35]</sup>, preferably thiazide or loop ones, because of their efficiency in reducing hyperkalemia<sup>[41]</sup>. In patients with a GFR < 30 mL/min, a loop diuretic is ideal, as thiazide diuretics are less effective<sup>[52]</sup>. Alternatively, other authors have initially chosen to suspend the use of ARBs and start diuretics to first control potassium levels and then restart RAAS blockade successfully<sup>[56]</sup>. However, the use of loop diuretics may lead to the deterioration of renal function because of intravascular volume depletion<sup>[20]</sup>. If challenges in managing hyperkalemia persist, it is recommended to discontinue medications that act on the RAAS<sup>[41]</sup>; however, new agents are being evaluated in the treatment of hyperkalemia. Sodium zirconium cyclosilicate, a selective cation exchanger, was tested in a multicenter study and led to a significant reduction in potassium levels at 48 h (approximately 0.5 to 0.7 mEq/L)<sup>[57]</sup>. However, the most promising drug to date is patiomer, a non-absorbed potassium binder, which has been tested in patients with hyperkalemia and with use of RAAS blockers. In a multicenter study, patiomer

was able to significantly reduce the level of potassium by approximately 1 mEq/L (mmol/L) in hyperkalemic patients using ACEIs or ARBs<sup>[58]</sup>.

## CONCLUSION

In clinical practice, it is common for patients with DM to present with hyperkalemia, especially if they are monitored for electrolytes. The literature states that the main cause of hyperkalemia in those patients, particularly those who already have diabetic microvascular complications (such as autonomic neuropathy), is HH. However, the recent literature on this topic is quite limited. Despite its pathophysiological importance, the diagnosis of HH is actually difficult to make because of the high frequency of concomitant comorbidities as well as the use of different medications and clinical variability. Clinically, HH is most often found in patients with DN, and the patients typically have asymptomatic, mild to moderate hyperkalemia. The prevalence of HH in the general diabetic population remains unclear, but it is believed to be underdiagnosed by physicians, including diabetologists. ACEIs and ARBs may precipitate hyperkalemia in a patient whose disease has not been recognized and increase the risk of severe hyperkalemia in patients with previously mild hyperkalemia. Although ACEIs and ARBs are considered to be essential for nephroprotection and are key drugs in the treatment of hypertension and DN, new treatment strategies are needed to achieve better effectiveness and control of potassium imbalances. Therefore, preventive actions should be routinely taken when treating such patients, including the proper evaluation of patients with initial borderline hyperkalemia to detect HH and the monitoring of patient potassium levels after initiating or modifying medications that block the RAAS. Undoubtedly, further studies are required to clarify critical issues regarding the syndrome of HH.

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## REFERENCES

- 1 **Raebl MA**, Ross C, Cheetham C, Petersen H, Saylor G, Smith DH, Wright LA, Roblin DW, Xu S. Increasingly restrictive definitions of hyperkalemia outcomes in a database study: effect on incidence estimates. *Pharmacoepidemiol Drug Saf* 2010; **19**: 19-25 [PMID: 19937982 DOI: 10.1002/pds.1882]
- 2 **Karet FE**. Mechanisms in hyperkalemic renal tubular acidosis. *J Am Soc Nephrol* 2009; **20**: 251-254 [PMID: 19193780 DOI: 10.1681/ASN.2008020166]
- 3 **Palmer BF**. A physiologic-based approach to the evaluation of a patient with hyperkalemia. *Am J Kidney Dis* 2010; **56**: 387-393 [PMID: 20493606 DOI: 10.1053/j.ajkd.2010.01.020]
- 4 **Root AW**. Disorders of aldosterone synthesis, secretion, and cellular function. *Curr Opin Pediatr* 2014; **26**: 480-486 [PMID: 24840884 DOI: 10.1097/MOP.0000000000000104]
- 5 **Cox M**, Sterns RH, Singer I. The defense against hyperkalemia:



- the roles of insulin and aldosterone. *N Engl J Med* 1978; **299**: 525-532 [PMID: 355876 DOI: 10.1056/NEJM197809072991007]
- 6 **Uribarri J**, Oh MS, Carroll HJ. Hyperkalemia in diabetes mellitus. *J Diabet Complications* 1990; **4**: 3-7 [PMID: 2141843]
- 7 **Lehnhardt A**, Kemper MJ. Pathogenesis, diagnosis and management of hyperkalemia. *Pediatr Nephrol* 2011; **26**: 377-384 [PMID: 21181208 DOI: 10.1007/s00467-010-1699-3]
- 8 **Watanabe T**, Nitta K. Transient hyporeninemic hypoaldosteronism in acute glomerulonephritis. *Pediatr Nephrol* 2002; **17**: 959-963 [PMID: 12432442 DOI: 10.1007/s00467-002-0984-1]
- 9 **DeFronzo RA**. Hyperkalemia and hyporeninemic hypoaldosteronism. *Kidney Int* 1980; **17**: 118-134 [PMID: 6990088]
- 10 **Phelps KR**, Lieberman RL, Oh MS, Carroll HJ. Pathophysiology of the syndrome of hyporeninemic hypoaldosteronism. *Metabolism* 1980; **29**: 186-199 [PMID: 6986536]
- 11 **Bojestig M**, Nystrom FH, Arnqvist HJ, Ludvigsson J, Karlberg BE. The renin-angiotensin-aldosterone system is suppressed in adults with Type 1 diabetes. *J Renin Angiotensin Aldosterone Syst* 2000; **1**: 353-356 [PMID: 11967822 DOI: 10.3317/jraas.2000.065]
- 12 **Nakamura R**, Saruta T, Yamagami K, Saito I, Kondo K, Matsuki S. Renin and the juxtaglomerular apparatus in diabetic nephropathy. *J Am Geriatr Soc* 1978; **26**: 17-21 [PMID: 618949]
- 13 **Schambelan M**, Sebastian A, Biglieri EG. Prevalence, pathogenesis, and functional significance of aldosterone deficiency in hyperkalemic patients with chronic renal insufficiency. *Kidney Int* 1980; **17**: 89-101 [PMID: 6990090]
- 14 **Hoskote SS**, Joshi SR, Ghosh AK. Disorders of potassium homeostasis: pathophysiology and management. *J Assoc Physicians India* 2008; **56**: 685-693 [PMID: 19086355]
- 15 **Mory PB**, Santos MC, Kater CE, Moisés RS. Maternally-inherited diabetes with deafness (MIDD) and hyporeninemic hypoaldosteronism. *Arq Bras Endocrinol Metabol* 2012; **56**: 574-577 [PMID: 23295301]
- 16 **Rodríguez Soriano J**. Renal tubular acidosis: the clinical entity. *J Am Soc Nephrol* 2002; **13**: 2160-2170 [PMID: 12138150]
- 17 **Eschmann E**, Beeler PE, Kaplan V, Schneemann M, Zünd G, Blaser J. Patient- and physician-related risk factors for hyperkalemia in potassium-increasing drug-drug interactions. *Eur J Clin Pharmacol* 2014; **70**: 215-223 [PMID: 24150532 DOI: 10.1007/s00228-013-1597-2]
- 18 **Montague BT**, Ouellette JR, Buller GK. Retrospective review of the frequency of ECG changes in hyperkalemia. *Clin J Am Soc Nephrol* 2008; **3**: 324-330 [PMID: 18235147 DOI: 10.2215/CJN.04611007]
- 19 **Onozaki A**, Katoh T, Watanabe T. Hyporeninemic hypoaldosteronism associated with Sjögren's syndrome. *Am J Med* 2002; **112**: 245-246 [PMID: 11893359]
- 20 **Takemoto F**, Ubara Y, Kaname S, Katori H, Sawa N, Hoshino J, Suwabe T, Higa Y, Nakanishi S, Nagata M, Ohashi K, Takaichi K. Hyporeninemic hypoaldosteronism from secondary amyloidosis. *Kidney Int* 2008; **74**: 542 [PMID: 18670413 DOI: 10.1038/ki.2008.14]
- 21 **Shaked Y**, Blau A, Shpilberg O, Samra Y. Hyporeninemic hypoaldosteronism associated with multiple myeloma: 11 years of follow-up. *Clin Nephrol* 1993; **40**: 79-82 [PMID: 8222376]
- 22 **Kim HW**, Lee DH, Lee SA, Koh G. A relationship between serum potassium concentration and insulin resistance in patients with type 2 diabetes mellitus. *Int Urol Nephrol* 2015; **47**: 991-999 [PMID: 25966806 DOI: 10.1007/s11255-015-1001-5]
- 23 **Liamis G**, Liberopoulos E, Barkas F, Elisaf M. Diabetes mellitus and electrolyte disorders. *World J Clin Cases* 2014; **2**: 488-496 [PMID: 25325058 DOI: 10.12998/wjcc.v2.i10.488]
- 24 **Grande Villoria J**, Macias Nunez JF, Miralles JM, De Castro del Pozo S, Tabernero Romo JM. Hyporeninemic hypoaldosteronism in diabetic patients with chronic renal failure. *Am J Nephrol* 1988; **8**: 127-137 [PMID: 3394721]
- 25 **Haas CS**, Pohlenz I, Lindner U, Muck PM, Arand J, Suefke S, Lehnert H. Renal tubular acidosis type IV in hyperkalemic patients—a fairy tale or reality? *Clin Endocrinol (Oxf)* 2013; **78**: 706-711 [PMID: 22891694 DOI: 10.1111/j.1365-2265.2012.04446.x]
- 26 **Ramírez E**, Rossignoli T, Campos AJ, Muñoz R, Zegarra C, Tong H, Medrano N, Borobia AM, Carcas AJ, Frias J. Drug-induced life-threatening potassium disturbances detected by a pharmacovigilance program from laboratory signals. *Eur J Clin Pharmacol* 2013; **69**: 97-110 [PMID: 22648277 DOI: 10.1007/s00228-012-1303-9]
- 27 **Medford-Davis L**, Rafique Z. Derangements of potassium. *Emerg Med Clin North Am* 2014; **32**: 329-347 [PMID: 24766936 DOI: 10.1016/j.emc.2013.12.005]
- 28 **Alpern RJ**, Sakhaee K. The clinical spectrum of chronic metabolic acidosis: homeostatic mechanisms produce significant morbidity. *Am J Kidney Dis* 1997; **29**: 291-302 [PMID: 9016905]
- 29 **Albareda MM**, Corcoy R. Reversible impairment of renal function associated with enalapril in a diabetic patient. *CMAJ* 1998; **159**: 1279-1281 [PMID: 9861226]
- 30 **Karmacharya P**, Poudel DR, Pathak R, Rettew A, Alweis R. Acute hyperkalemia leading to flaccid paralysis: a review of hyperkalemic manifestations. *J Community Hosp Intern Med Perspect* 2015; **5**: 27993 [PMID: 26091666 DOI: 10.3402/jchimp.v5.27993]
- 31 **Annane D**, Cariou A, Maxime V, Azoulay E, D'honneur G, Timsit JF, Cohen Y, Wolf M, Fartoukh M, Adrie C, Santré C, Bollaert PE, Mathonet A, Amathieu R, Tabah A, Clec'h C, Mayaux J, Lejeune J, Chevret S. Corticosteroid treatment and intensive insulin therapy for septic shock in adults: a randomized controlled trial. *JAMA* 2010; **303**: 341-348 [PMID: 20103758 DOI: 10.1001/jama.2010.2]
- 32 **Abraham SC**, Bhagavan BS, Lee LA, Rashid A, Wu TT. Upper gastrointestinal tract injury in patients receiving kayexalate (sodium polystyrene sulfonate) in sorbitol: clinical, endoscopic, and histopathologic findings. *Am J Surg Pathol* 2001; **25**: 637-644 [PMID: 11342776]
- 33 **Low PA**, Tomalia VA. Orthostatic Hypotension: Mechanisms, Causes, Management. *J Clin Neurol* 2015; **11**: 220-226 [PMID: 26174784 DOI: 10.3988/jcn.2015.11.3.220]
- 34 **El-Haddad B**, Reule S, Drawz PE. Dual Renin-Angiotensin-Aldosterone System Inhibition for the Treatment of Diabetic Kidney Disease: Adverse Effects and Unfulfilled Promise. *Curr Diab Rep* 2015; **15**: 70 [PMID: 26275442 DOI: 10.1007/s11892-015-0640-3]
- 35 **Sebastian A**, Schambelan M, Sutton JM. Amelioration of hyperchloremic acidosis with furosemide therapy in patients with chronic renal insufficiency and type 4 renal tubular acidosis. *Am J Nephrol* 1984; **4**: 287-300 [PMID: 6524600]
- 36 **Chimori K**, Miyazaki S, Kosaka J, Sakanaka A, Yasuda K, Miura K. The significance of autonomic neuropathy in the elevation of inactive renin in diabetes mellitus. *Clin Exp Hypertens A* 1987; **9**: 1-18 [PMID: 3555891]
- 37 **Yamaguchi M**. [A study on the pathogenesis of hyporeninemia in diabetics]. *Nihon Naibunpi Gakkai Zasshi* 1994; **70**: 573-584 [PMID: 7958107]
- 38 **Castellino P**, Simonson DC, DeFronzo RA. Adrenergic modulation of potassium metabolism during exercise in normal and diabetic humans. *Am J Physiol* 1987; **252**: E68-E76 [PMID: 3544863]
- 39 **Davis JO**, Freeman RH. Mechanisms regulating renin release. *Physiol Rev* 1976; **56**: 1-56 [PMID: 1108062]
- 40 **Sato A**. The necessity and effectiveness of mineralocorticoid receptor antagonist in the treatment of diabetic nephropathy. *Hypertens Res* 2015; **38**: 367-374 [PMID: 25762415 DOI: 10.1038/hr.2015.19]
- 41 **Palmer BF**. Managing hyperkalemia caused by inhibitors of the renin-angiotensin-aldosterone system. *N Engl J Med* 2004; **351**: 585-592 [PMID: 15295051 DOI: 10.1056/NEJMra035279]
- 42 **Arruda JA**, Battle DC, Sehj JT, Roseman MK, Baronowski RL, Kurtzman NA. Hyperkalemia and renal insufficiency: role of selective aldosterone deficiency and tubular unresponsiveness to aldosterone. *Am J Nephrol* 1981; **1**: 160-167 [PMID: 6758577]
- 43 **Shier DN**, Kusano E, Stoner GD, Franco-Saenz R, Mulrow PJ. Production of renin, angiotensin II, and aldosterone by adrenal explant cultures: response to potassium and converting enzyme inhibition. *Endocrinology* 1989; **125**: 486-491 [PMID: 2544410 DOI: 10.1210/endo-125-1-486]

- 44 **Palmer SC**, Mavridis D, Navarese E, Craig JC, Tonelli M, Salanti G, Wiebe N, Ruospo M, Wheeler DC, Strippoli GF. Comparative efficacy and safety of blood pressure-lowering agents in adults with diabetes and kidney disease: a network meta-analysis. *Lancet* 2015; **385**: 2047-2056 [PMID: 26009228 DOI: 10.1016/S0140-6736(14)62459-4]
- 45 **Lee BH**, Kang HG, Choi Y. Hyporeninemic hypoaldosteronism in a child with chronic kidney disease--is this condition renoprotective? *Pediatr Nephrol* 2009; **24**: 1771-1772 [PMID: 19189133 DOI: 10.1007/s00467-009-1121-1]
- 46 **Raebel MA**, Ross C, Xu S, Roblin DW, Cheetham C, Blanchette CM, Saylor G, Smith DH. Diabetes and drug-associated hyperkalemia: effect of potassium monitoring. *J Gen Intern Med* 2010; **25**: 326-333 [PMID: 20087674 DOI: 10.1007/s11606-009-1228-x]
- 47 **Fried LF**, Emanuele N, Zhang JH, Brophy M, Conner TA, Duckworth W, Leehey DJ, McCullough PA, O'Connor T, Palevsky PM, Reilly RF, Seliger SL, Warren SR, Watnick S, Peduzzi P, Guarino P. Combined angiotensin inhibition for the treatment of diabetic nephropathy. *N Engl J Med* 2013; **369**: 1892-1903 [PMID: 24206457 DOI: 10.1056/NEJMoa1303154]
- 48 **Mann JF**, Schmieder RE, McQueen M, Dyal L, Schumacher H, Pogue J, Wang X, Maggioni A, Budaj A, Chaithiraphan S, Dickstein K, Keltai M, Metsärinne K, Oto A, Parkhomenko A, Piegas LS, Svendsen TL, Teo KK, Yusuf S. Renal outcomes with telmisartan, ramipril, or both, in people at high vascular risk (the ONTARGET study): a multicentre, randomised, double-blind, controlled trial. *Lancet* 2008; **372**: 547-553 [PMID: 18707986 DOI: 10.1016/S0140-6736(08)61236-2]
- 49 **Parving HH**, Brenner BM, McMurray JJ, de Zeeuw D, Haffner SM, Solomon SD, Chaturvedi N, Persson F, Desai AS, Nicolaidis M, Richard A, Xiang Z, Brunel P, Pfeffer MA. Cardiorenal end points in a trial of aliskiren for type 2 diabetes. *N Engl J Med* 2012; **367**: 2204-2213 [PMID: 23121378 DOI: 10.1056/NEJMoa1208799]
- 50 **Seliger SL**, Fried LF. Serum potassium in dual renin-angiotensin-aldosterone system blockade. *Clin J Am Soc Nephrol* 2014; **9**: 219-221 [PMID: 24408119 DOI: 10.2215/CJN.12411213]
- 51 **Putcha N**, Allon M. Management of hyperkalemia in dialysis patients. *Semin Dial* 2007; **20**: 431-439 [PMID: 17897250 DOI: 10.1111/j.1525-139X.2007.00312.x]
- 52 **Palmer BF**, Clegg DJ. Electrolyte and Acid-Base Disturbances in Patients with Diabetes Mellitus. *N Engl J Med* 2015; **373**: 548-559 [PMID: 26244308 DOI: 10.1056/NEJMra1503102]
- 53 **Cushman WC**, Evans GW, Byington RP, Goff DC, Grimm RH, Cutler JA, Simons-Morton DG, Basile JN, Corson MA, Probstfield JL, Katz L, Peterson KA, Friedewald WT, Buse JB, Bigger JT, Gerstein HC, Ismail-Beigi F. Effects of intensive blood-pressure control in type 2 diabetes mellitus. *N Engl J Med* 2010; **362**: 1575-1585 [PMID: 20228401 DOI: 10.1056/NEJMoa1001286]
- 54 **Cada DJ**, Ingram KT, Levien TL, Baker DE. Canagliflozin. *Hosp Pharm* 2013; **48**: 855-867 [PMID: 24421439 DOI: 10.1310/hpj4810-855]
- 55 **Inada M**, Iwasaki K, Imai C, Hashimoto S. Hyperpotassemia and bradycardia in a bedridden elderly woman with selective hypoaldosteronism associated with low renin activity. *Intern Med* 2010; **49**: 307-313 [PMID: 20154436]
- 56 **van Nieuwkoop C**, Ijpelaar DH, Bolk JH. Treating proteinuria in a diabetic patient despite hyperkalaemia due to hyporeninaemic hypoaldosteronism. *Neth J Med* 2007; **65**: 75-77 [PMID: 17379933]
- 57 **Packham DK**, Rasmussen HS, Lavin PT, El-Shahawy MA, Roger SD, Block G, Qunibi W, Pergola P, Singh B. Sodium zirconium cyclosilicate in hyperkalemia. *N Engl J Med* 2015; **372**: 222-231 [PMID: 25415807 DOI: 10.1056/NEJMoa1411487]
- 58 **Weir MR**, Bakris GL, Bushinsky DA, Mayo MR, Garza D, Stasiv Y, Wittes J, Christ-Schmidt H, Berman L, Pitt B. Patiromer in patients with kidney disease and hyperkalemia receiving RAAS inhibitors. *N Engl J Med* 2015; **372**: 211-221 [PMID: 25415805 DOI: 10.1056/NEJMoa1410853]

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Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China

Telephone: +86-10-85381891

Fax: +86-10-85381893

E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)

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## Low serum amylase and obesity, diabetes and metabolic syndrome: A novel interpretation

Kei Nakajima

Kei Nakajima, Department of Metabolism, Kuki General Hospital, Saitama 346-8530, Japan

Kei Nakajima, Division of Clinical Nutrition, Department of Medical Dietetics, Faculty of Pharmaceutical Sciences, Josai University, Saitama 350-0295, Japan

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**Correspondence to:** Kei Nakajima, MD, PhD, Department of Metabolism, Kuki General Hospital, 418-1 Kamihayami, Kuki, Saitama 346-8530, Japan. [keinaka@josai.ac.jp](mailto:keinaka@josai.ac.jp)  
Telephone: +81-480-260033  
Fax: +81-480-260033

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### Abstract

For the last decade, low serum amylase (hypoamylasemia) has been reported in certain common cardiometabolic conditions such as obesity, diabetes (regardless of type), and metabolic syndrome, all of

which appear to have a common etiology of insufficient insulin action due to insulin resistance and/or diminished insulin secretion. Some clinical studies have shown that salivary amylase may be preferentially decreased in obese individuals, whereas others have revealed that pancreatic amylase may be preferentially decreased in diabetic subjects with insulin dependence. Despite this accumulated evidence, the clinical relevance of serum, salivary, and pancreatic amylase and the underlying mechanisms have not been fully elucidated. In recent years, copy number variations (CNVs) in the salivary amylase gene (*AMY1*), which range more broadly than the pancreatic amylase gene (*AMY2A* and *AMY2B*), have been shown to be well correlated with salivary and serum amylase levels. In addition, low CNV of *AMY1*, indicating low salivary amylase, was associated with insulin resistance, obesity, low taste perception/satiety, and postprandial hyperglycemia through impaired insulin secretion at early cephalic phase. In most populations, insulin-dependent diabetes is less prevalent (minor contribution) compared with insulin-independent diabetes, and obesity is highly prevalent compared with low body weight. Therefore, obesity as a condition that elicits cardiometabolic diseases relating to insulin resistance (major contribution) may be a common determinant for low serum amylase in a general population. In this review, the novel interpretation of low serum, salivary, and pancreas amylase is discussed in terms of major contributions of obesity, diabetes, and metabolic syndrome.

**Key words:** Serum amylase; Salivary; Pancreas; Diabetes; Metabolic syndrome; Obesity; *AMY1*; *AMY2*; Insulin resistance

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**Core tip:** Low serum amylase was believed to occur in uncommon conditions such as type 1 diabetes, advanced chronic pancreatitis, and cystic fibrosis.

However, in the last decade, low serum amylase has been observed in more common conditions related with insulin resistance than was previously believed. In this review, a novel interpretation for low serum, salivary, and pancreatic amylase is discussed, particularly in terms of the cardiometabolic conditions of obesity, diabetes, and metabolic syndrome.

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## INTRODUCTION

Traditionally, the level of serum amylase has been commonly measured to determine the presence of acute pancreatitis and biliary tract disease in primary clinical settings<sup>[1-3]</sup>. In contrast, most physicians seldom measure it to determine the degree of advanced chronic pancreatitis, which eventually results in secondary diabetes concomitant with weight loss, lipid diarrhea, and malnutrition<sup>[4-6]</sup>. The former condition predisposes to higher serum amylase (but not necessarily), whereas the latter condition lowers serum amylase. While some cancers, such as lung, ovarian, and colon cancer and myeloma, often produce amylase and increase serum amylase<sup>[7-9]</sup>, such increased serum amylase may be exceptional in a general population. By contrast, low serum amylase has been empirically known in diabetic patients particularly with insulin-dependent diabetes (primarily type 1 diabetes), although the clinical relevance and precise underlying mechanism are not fully understood<sup>[10-15]</sup>.

In earlier clinical studies, conflicting results were reported regarding the level of serum amylase in diabetic patients, possibly because a precise measurement for serum amylase had not then been established. In recent decades, however, the measurement of serum and its isoforms has been stably performed in clinical laboratories with several methods including electrophoresis, inhibitor method and antibody method<sup>[16]</sup>. Because serum amylase generally consists of salivary and pancreatic amylase at almost equal proportion, *i.e.*, 1:1<sup>[17]</sup>, abnormal levels of both or one of the two isoforms affect the level of total serum amylase.

Low serum amylase was believed to occur in uncommon conditions such as type 1 diabetes, advanced chronic pancreatitis, and cystic fibrosis<sup>[1-3,10-15,18,19]</sup> (minor contribution) (Table 1). However, in the past decade, low serum amylase has been observed in more common conditions (major contribution) than was previously believed. In this review, a novel interpretation for low serum, salivary, and pancreatic amylase is discussed,

**Table 1** Traditional and novel interpretations for low serum amylase

	Ref.
Traditional (minor contributions)	
Type 1 diabetes (juvenile diabetes)	[1,2,10-15]
Advanced chronic pancreatitis	[3-6]
Type 2 diabetes with insulin dependence	[14]
Cystic fibrosis	[18,19]
Novel (major contributions)	
Obesity	[35-38]
Insulin resistance (high HOMA-R)	[44]
Metabolic Syndrome	[38,41,42]
Type 2 diabetes with insufficient insulin action	[38,42]
Diabetic ketoacidosis	[13,23]
Non-alcoholic fatty liver disease	[40,43]
Smoking	[38,68-70]
Heavy alcohol drinking	[46,63]
Low CNVs of AMY1	[54,60,61]

HOMA-R: Homeostasis model of insulin resistance; CNV: Copy number variations; AMY1: Salivary amylase gene.

particularly in terms of the cardiometabolic conditions of obesity, diabetes, and metabolic syndrome (MetS).

## TRADITIONAL INTERPRETATION

### **Specific etiologies related to low serum amylase (minor determinant for low serum amylase)**

In the general population, the cause of acute pancreatitis is mainly high alcohol intake and/or high serum triglycerides, both of which injure the pancreas<sup>[1-6]</sup>. Consequently, transient and acute increases (from several to ten times beyond the upper normal level) in serum amylase concentration may occur as a result of destruction of acinar cells in the pancreas. However, repeated acute pancreatitis eventually results in exhausted acinar cells and restricted flow of enzymes from pancreas parenchyma into the circulation<sup>[4-6]</sup>, in turn leading to low serum amylase due to low pancreatic amylase. Secondary diabetes also develops because of the destruction of  $\beta$ -cells in the course of chronic pancreatitis. In addition, low serum amylase has been observed in patients with cystic fibrosis concomitant with pancreatic insufficiency<sup>[18,19]</sup>.

Around 20% of patients with diabetic ketoacidosis develop hyperamylasemia<sup>[20-22]</sup>. In this author's opinion, however, because diabetic ketoacidosis is accompanied by severely insufficient insulin action, theoretically serum amylase should be decreased in such conditions, at least before insulin therapy is initiated.

Consistently with this, Yokoyama *et al*<sup>[13]</sup> and Somogyi *et al*<sup>[23]</sup> found that serum amylase activity was reduced at onset of the disease before treatment with insulin. The author of the current review and collaborators, have frequently observed low serum amylase in diabetic ketoacidosis before treatment with insulin in clinical settings (unpublished data). The unexpectedly high serum amylase in previous

studies<sup>[20-22]</sup> may be explained by the fact that diabetic ketoacidosis involves numerous etiologies that can contribute to high serum amylase: Acute pancreatitis (mild to moderate grades)<sup>[22]</sup> including hypertriglyceridemic pancreatitis<sup>[24]</sup>, renal dysfunction, and dehydration, all of which increase serum amylase.

Meanwhile, low serum amylase has been empirically observed in clinical settings in patients with type 1 diabetes, type 2 diabetes with insulin dependence, or advanced overt pancreatitis<sup>[10-15]</sup>. The action of insulin is critical for the production of pancreatic amylase<sup>[25,26]</sup>. A common etiology in these conditions may be depleted secretion of insulin from the pancreas. However, patients with these specific conditions are minor populations compared with diabetic patients with insulin independence.

The clinical relevance of salivary amylase has been focused on diseases of the salivary glands, sympathetic nerve system, and oral health. Under physiological conditions, secretion and activation of salivary amylase, *i.e.*, hyperamylasemia, is reportedly stimulated by psychosocial stress<sup>[27,28]</sup>. Many  $\alpha$ -amylase inhibitors, which are often extracted from plants, have also been intensively investigated in terms of carbohydrate digestion and diabetic treatment<sup>[29,30]</sup>. Unfortunately, however, few clinical studies have reported low salivary amylase.

Dentists and investigators who work with, or are interested in, oral care have addressed the etiology of low salivary amylase<sup>[31,32]</sup>. Although insulin may also exert its action on the production of salivary amylase in the salivary glands<sup>[33,34]</sup>, physicians have paid little attention to the issue. Therefore, the clinical relevance of low salivary amylase has not been elucidated, particularly in cardiometabolic conditions.

## NOVEL INTERPRETATION

### ***Relationship with cardiometabolic conditions of obesity and obesity-related disease (major determinants for low serum amylase)***

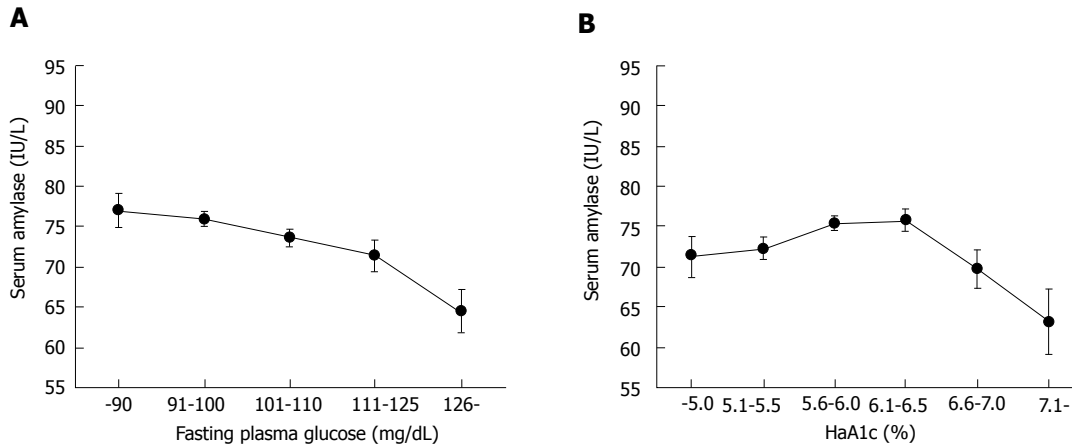
An early animal study by Schneeman *et al*<sup>[35]</sup> showed that pancreatic amylase activity was reduced in obese rats but remained elevated in lean rats. An early clinical study of healthy young men aged 19 to 22 years by Kondo *et al*<sup>[36]</sup> showed that serum pancreatic amylase and trypsin, but not lipase, were reduced in obese subjects ( $n = 85$ ) compared with lean subjects ( $n = 75$ ). The reduction in serum pancreatic amylase was significantly improved by a weight loss program over 6 mo, and remained improved for a further 10 mo. No such trend was observed in pancreatic trypsin. To the best of this author's knowledge, these are the first studies to show an inverse relationship between obesity and serum pancreatic amylase. However, in the clinical study<sup>[36]</sup>, the sample size was relatively small and relevant confounding factors including smoking, alcohol intake, exercise, and kidney function were

not adjusted for in the analysis. Another small clinical study in children ( $n = 58$ ) showed that obese boys ( $n = 29$ ) presented a significantly lower salivary amylase concentration than control boys<sup>[37]</sup>. Except for these early studies<sup>[35-37]</sup>, no clinical studies have investigated the relationship between serum amylase and obesity and obesity-related conditions.

In our previous cross-sectional ( $n = 2425$ ) and longitudinal ( $n = 571$ ) studies during the last decade<sup>[38]</sup>, low serum (total) amylase ( $\leq 57$  IU/L) was significantly associated with MetS, diabetes (mostly type 2 diabetes), and remained significant even after adjustment for relevant confounding factors including age, sex, smoking, alcohol drinking, and regular exercise, pharmacotherapies, and kidney function assessed by estimated glomerular filtration rate (eGFR). In this study, body mass index (BMI) was the factor most associated with serum amylase<sup>[38]</sup>. Furthermore, low serum amylase was associated with non-alcoholic fatty liver disease (NAFLD), a hepatic manifestation of MetS and insulin resistance<sup>[39]</sup>, in asymptomatic adults independently of relevant confounding factors<sup>[40]</sup>. The results of these epidemiological studies<sup>[38,40]</sup> were subsequently confirmed in other large Asian populations<sup>[41-43]</sup>. Furthermore, in our previous study of asymptomatic subjects not being treated for diabetes<sup>[44]</sup>, a homeostasis model assessment of insulin resistance, plasma insulin levels at fasting and at 60 min in the 75 g oral glucose tolerance test were significantly associated with low serum amylase ( $< 60$  IU/L) after adjustment for relevant confounding factors including BMI, although the sample size was small ( $n = 54$ ).

These results suggest that low serum amylase is observed in not only rare conditions of insulin depletion (minor contribution) but also in common cardiometabolic conditions such as MetS, type 2 diabetes, or NAFLD (major contribution). Obesity, as a condition associated with various cardiometabolic diseases concomitant with insulin resistance, may be a major determinant for low serum amylase in the general population (a novel interpretation). A clinical study in hospitalized patients by Curd *et al*<sup>[45]</sup> showed that hypoamylasemia was associated with cystic fibrosis, hypertriglyceridemia and use of the antibiotic gentamicin, besides diabetes mellitus. Although cystic fibrosis and use of gentamicin may be uncommon, hypertriglyceridemia is rather common.

Williams *et al*<sup>[46]</sup> mentioned in an early review article that insulin is necessary for normal acinar function and that endogenous insulin potentiates zymogen release. However, exogenous insulin supplementation can improve low serum amylase in type 1 diabetes<sup>[13]</sup>. Schneeman *et al*<sup>[35]</sup> proposed in an animal study that insulin resistance may prevent the potentiating effect of insulin on amylase synthesis, leading to lower amylase levels. Early clinical studies have also shown that serum pancreatic amylase was closely related to C-peptide concentration and pancreatic  $\beta$ -cell function<sup>[13,14]</sup>. One



**Figure 1 Relationship between serum amylase and fasting plasma glucose (A) and glycosylated hemoglobin (national glycohemoglobin standardization program) (B).** Fasting plasma glucose of 90 mg/dL corresponds to HbA1c of 5.0% NGSP, as determined by linear regression analysis between FPG and HbA1c<sup>[47]</sup>. FPG: Fasting plasma glucose; NGSP: National glycohemoglobin standardization program; HbA1c: Glycosylated hemoglobin.

would therefore expect serum amylase to be reduced in obese and diabetic subjects.

Regarding the relationship between diabetes and serum amylase, it is noteworthy that serum amylase levels are not linearly correlated with HbA1c values in the general population including healthy individuals and diabetic patients, although fasting plasma glucose was negatively and linearly correlated with serum amylase<sup>[47]</sup>. In this study, serum amylase showed an inverse U-shaped relationship with HbA1c categories (Figure 1). Unexpectedly, serum amylase level was highest in subjects with HbA1c of 5.6%-6.5%. We experienced a similar result in an entirely different Japanese population (unpublished data). These findings may be consistent with the results of an early study by Dandona *et al*<sup>[14]</sup>, which showed no significant correlation between HbA1c and pancreatic amylase activity. The discrepancy between HbA1c and fasting plasma glucose may be owing to the presence of postprandial hyperglycemia, *i.e.*, impaired glucose tolerance, a common finding in obese individuals<sup>[48,49]</sup>, primarily related to HbA1c only. Additionally, hyperinsulinemia induced by insulin resistance for the maintenance of euglycemia, a common finding in early type 2 diabetes, may increase pancreatic amylase production.

After progression to overt diabetes with HbA1c over 6.5%, insulin resistance is not managed by hyperinsulinemia and insulin secretion begins to decline, resulting in a corresponding decrease in pancreatic and/or salivary amylase. Therefore, a linear relationship between HbA1c and serum amylase was observed in the data when only overt diabetic patients were studied<sup>[13,47]</sup>.

Taken together, these results suggest that the relationship between serum amylase and diabetes and obesity is an exocrine-endocrine interrelationship, which in turn may contribute to the feedback system in energy homeostasis.

## SALIVARY AMYLASE

While vertebrate animals express amylase in the pancreas, its expression in the salivary gland is limited to some primates and other herbivores and omnivores<sup>[50]</sup>. Carnivores (domesticated dogs and cats) do not have salivary amylase, whereas many herbivores (including goats, cows, horse, koala, rabbit, and elephant) do. Conversely, most omnivores, including humans, have considerable amounts of salivary amylase. Salivary amylase is higher in humans compared with many other animals including ape species, suggesting a dietary shift in the direction of high starch content during evolution<sup>[50,51]</sup>.

Salivary amylase can affect an individual's oral sensory properties, in turn altering the threshold of satiety and appetite. While amylase expression particularly in salivary glands may be roughly determined by genetic regulation, high amylase levels can be induced by carbohydrate-rich diets passed on over generations. This hypothesis warrants further study.

Postprandial plasma glucose concentrations after ingestion of a 50 g starch solution were significantly higher in healthy nonobese adults with low salivary amylase than in those with high salivary amylase<sup>[52]</sup>. High salivary amylase activity is associated with a rapid insulin response accompanied by a swift reduction in blood glucose levels following starch ingestion. A plausible explanation is the response of insulin secretion at the early cephalic phase.

## Genetic regulation

Genetic regulation is likely to play a key role in the primary determination of salivary amylase<sup>[53,54]</sup>. In newborns the predominant amylase isozymes seen in the urine are of salivary origin and later both salivary and pancreas, which increases during development. *AMY1* is expressed as early as 18 wk of gestation and salivary amylase gradually increases during



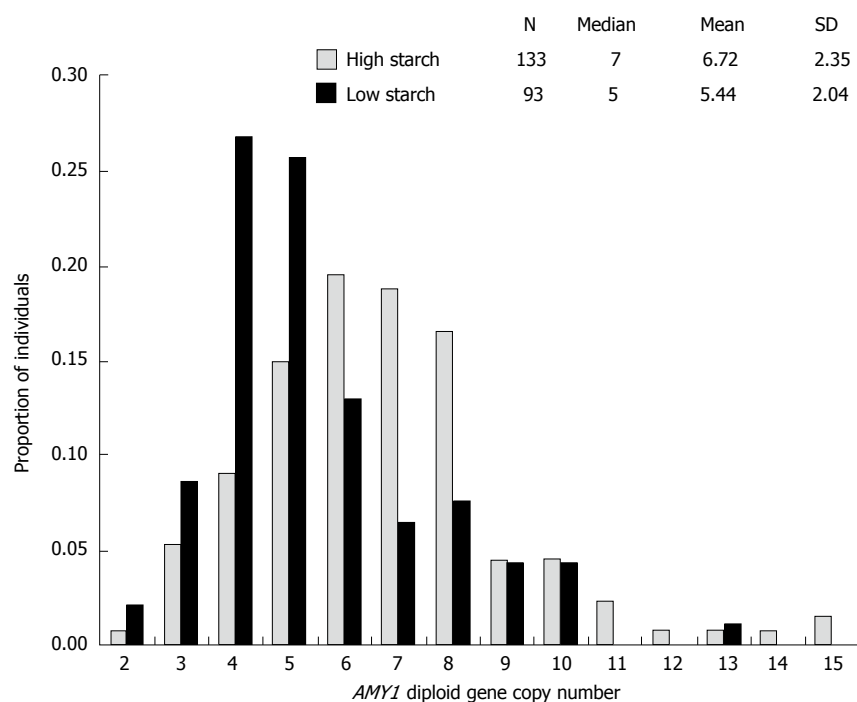


Figure 2 Copy number variations of the salivary amylase gene (*AMY1*) in relation to dietary starch<sup>[58]</sup>.

development, as the total amylase activity approaches adult values.

In recent years, several studies have reported that serum and salivary amylase was significantly correlated with copy number variations (CNVs) of salivary amylase gene (*AMY1*). Moreover, CNV of *AMY1* was inversely associated with BMI, insulin resistance, and glucose tolerance<sup>[52,55–57]</sup>. CNVs seem to be higher in humans, particularly in American Europeans and Japanese, who relied on a starch-rich diet in the remote past<sup>[58]</sup> (Figure 2). According to Falchi *et al.*<sup>[55]</sup>, CNV of *AMY1* had a stronger association with BMI than polymorphisms in *FTO*, although conflicting results exist<sup>[59]</sup>. These findings suggest a genetic link between carbohydrate metabolism and obesity, possibly also involving gut microbiota<sup>[60]</sup>.

A clinical study in Mexico suggests putative benefits of a high number of *AMY1* copies (and related production of salivary amylase) on obesity and energy metabolism in children<sup>[61]</sup>. Furthermore, a clinical study in Finland showed that low CNV of *AMY1* was associated with early-onset female obesity<sup>[62]</sup>. In this context, it is possible that individualized carbohydrate diets according to CNV of *AMY1* may help prevent obesity and type 2 diabetes.

Compared with *AMY1*, the relation of *AMY2* with cardiometabolic conditions is equivocal. In humans, the variation of CNVs of *AMY1* was wider than those of *AMY2*<sup>[57,59]</sup>. Furthermore, CNV of *AMY1* was independent of those of *AMY2*, which showed no association with BMI<sup>[59]</sup>. Meanwhile, in domesticated dogs for instance, pancreatic amylase not salivary amylase likely contributes to total amylase<sup>[50,63]</sup>. However, even in such

animals, the variation of CNV of *AMY2B* was estimated to explain only 14.8% of the variance in amylase activity, indicating that additional factors may explain the majority of the variation<sup>[63]</sup>.

## OTHER CONDITIONS AFFECTING AMYLASE LEVELS

### *Alcohol intake, smoking, exercise, stress, and other factors*

Other common conditions besides obesity-related conditions have been reported (Table 1). Alcohol consumption may affect the serum amylase level independently of BMI<sup>[47,64,65]</sup>. This may occur *via* damage of pancreatic tissue, *i.e.*, chronic pancreatitis, and reduced salivary amylase<sup>[66]</sup>. However, the underlying mechanism may be complicated because the effect of alcohol on glucose homeostasis can differ according to the quantity consumed<sup>[67]</sup>, age, and lifestyle<sup>[68]</sup>. Low serum amylase was also observed in smokers compared with nonsmokers<sup>[69–71]</sup>. In contrast, high serum amylase has been observed in individuals who exercise regularly<sup>[72]</sup> and in high-performance long distance runners<sup>[73]</sup>. Because both smoking and fitness have a substantial impact on insulin action, these results may be explained from the view of insulin sensitivity<sup>[74]</sup>. Furthermore, low serum pancreatic enzyme levels predict mortality and are associated with malnutrition-inflammation<sup>[75]</sup>, although the underlying mechanism remains unknown.

A recent study by Shimizu *et al.*<sup>[76]</sup> showed that circulating pancreatic amylase was higher in female subjects with O blood type than those with A blood

type (lower pancreatic amylase in A blood type). Serum total amylase was also higher (but not statistically significant) in O blood type in both sexes. Coincidentally, we confirmed a similar finding of lower serum amylase in A blood type relative to O blood type in a general Japanese adult population ( $n = 1185$ ), although no ABO blood types were associated with hyperglycemia ( $\text{HbA1c} \geq 5.7\%$  and/or pharmacotherapy for diabetes)<sup>[77]</sup>. Meanwhile, some clinical studies<sup>[78,79]</sup> have shown that people with the O blood type had a lower risk of developing type 2 diabetes compared with other blood types. Therefore, it is possible that unknown and unquantified factors including CNV of *AMY1* and prevalence of Rhesus factor in the study may also contribute to the relationship among ABO blood types, serum amylase, and impaired glucose metabolism. Together with the putative lower prevalence of pancreatic cancers in individuals with O blood type<sup>[80-82]</sup>, this indicates a possible relationship between ABO blood type, which is under strict genetic regulation, and susceptibility to pancreatic disease.

### Eating disorders

Intriguingly, elevated serum amylase has been sporadically observed in a series of eating disorders. Anorexia nervosa (AN) and bulimia nervosa (BN) are two major eating disorders with a complex relationship with abnormal physical conditions such as severe weight loss, binge eating, frequent vomiting or endocrine disorders<sup>[83,84]</sup>. Several studies have shown that serum amylase levels were significantly elevated in patients with BN<sup>[85-87]</sup>. It is likely that vomiting, rather than binge behavior, increases amylase in BN patients<sup>[87]</sup>. Frequent vomiting may also be associated with enlarged submandibular and parotid glands. It was also confirmed that hyperamylasemia in patients with AN or BN was caused by increased salivary-type amylase activity<sup>[85]</sup>. Consequently, these results suggest that the direct effect on elevated serum amylase may be primarily enlarged salivary glands. Conversely, it is also possible that concomitant low body weight or depleted energy storage, common findings in AN, cause the increased serum amylase. This needs to be studied further.

## CAUTION IN INTERPRETING SERUM AMYLASE

Psychosocial stress contributes to elevated salivary amylase even in a healthy population<sup>[27,28]</sup>, which likely leads to elevated total serum amylase. However, whether psychosocial stress has a long-term effect on serum amylase has not been confirmed in clinical studies. Investigators should pay attention to the mental and physical conditions in their patients when measuring salivary and serum amylase levels. Kidney function is also a crucial modifier that affects the

clearance of circulating amylase in the blood<sup>[38,88]</sup>. Serum amylase is expected to be elevated in patients with renal dysfunction, and is a permanent phenomenon. Marked serum amylase elevation is observed in patients with only chronic kidney disease (CKD)<sup>[89]</sup>. While renal dysfunction should be kept in mind when high amylase levels are detected, the conditions of low serum amylase can be hidden and thus overlooked in patients with CKD and renal dysfunction, because lower amylase can be converted to normal amylase as a result of diminished clearance. GFR, for instance by the use of inulin, is not measured in usual clinical settings, so eGFR should be at least considered as a relevant confounding factor in the analysis of serum amylase. Nevertheless, it is unknown whether hyperfiltration, which is often observed in early diabetes, lowers serum amylase.

Some pharmacotherapies, particularly against diabetes, for instance dipeptidyl peptidase-4 inhibitors and glucagon-like peptide 1 receptor agonists, reportedly increase serum amylase. Several investigators<sup>[90-92]</sup> have recommended caution when starting incretin therapy to avoid pancreatitis. However, theoretically, a mild increase in serum amylase levels within the normal range or from low to normal levels can represent a potential beneficial effect of incretin therapy on glucose homeostasis especially in individuals with appreciable weight loss. Physicians may withdraw incretin therapy in patients with a slight increase in serum amylase of 10-20 IU/mL, for example, interpreting this as a sign of pancreatitis. Increase in serum amylase, particularly from low to normal levels, may reflect improved glucose homeostasis rather than acute pancreatitis. However, it remains pivotal to accumulate further data to investigate the clinical relevance of increases in pancreatic enzymes during incretin therapy.

For several decades, some clinical studies have revealed that  $\alpha$ -amylase inhibitors, most of which are extracted from plants (clinically unavailable), can improve postprandial hyperglycemia<sup>[93]</sup> and obesity<sup>[94,95]</sup>. It is unclear whether  $\alpha$ -amylase inhibitors would be truly effective for preventing diabetes and obesity if clinically available. Furthermore, while acarbose, an  $\alpha$ -glucosidase inhibitor that also inhibits  $\alpha$ -amylase, is available, such agents may be ineffective in obese subjects who have already low serum amylase.

## CONCLUSION

Collectively, low serum amylase may reflect a manifestation of insufficient insulin action regardless of cause including insufficient pancreatic insulin secretion and/or systemic insulin resistance. Unfortunately, the cut-off point for low serum amylase has not been defined, primarily because of the lack of a concept for low serum amylase and the differences in assay methods. Unlike minor contributions, major contributions for low serum

amylase include common cardiometabolic conditions such as obesity, MetS, and type 2 diabetes, which are all increasing in incidence worldwide. Although genetic regulation may have a substantial impact on primary salivary amylase, whether epigenetic background and individual diet can alter salivary amylase and thus affect serum amylase is unclear, and requires further investigation.

## REFERENCES

- 1 Kameya A, Hayakawa T, Noda A, Kondo T. Differential determination of serum isoamylase using an amylase inhibitor and its clinical application. *Am J Gastroenterol* 1985; **80**: 54-59 [PMID: 3966456]
- 2 Garrison R. Amylase. *Emerg Med Clin North Am* 1986; **4**: 315-327 [PMID: 2422011]
- 3 Pieper-Bigelow C, Strocchi A, Levitt MD. Where does serum amylase come from and where does it go? *Gastroenterol Clin North Am* 1990; **19**: 793-810 [PMID: 1702756]
- 4 Worning H. Chronic pancreatitis: pathogenesis, natural history and conservative treatment. *Clin Gastroenterol* 1984; **13**: 871-894 [PMID: 6386243]
- 5 Giger U, Stanga Z, DeLegge MH. Management of chronic pancreatitis. *Nutr Clin Pract* 2004; **19**: 37-49 [PMID: 16215095 DOI: 10.1177/011542650401900137]
- 6 Waljee AK, Dimagno MJ, Wu BU, Schoenfeld PS, Conwell DL. Systematic review: pancreatic enzyme treatment of malabsorption associated with chronic pancreatitis. *Aliment Pharmacol Ther* 2009; **29**: 235-246 [PMID: 19035969 DOI: 10.1111/j.1365-2036.2008.03885.x]
- 7 Flood JG, Schuerch C, Dorazio RC, Bowers GN. Marked hyperamylasemia associated with carcinoma of the lung. *Clin Chem* 1978; **24**: 1207-1212 [PMID: 207468]
- 8 Ebisawa S, Yamazaki S, Yasuo M, Urushihata K, Tsushima K, Hanaoka M, Koizumi T, Fujimoto K, Kubo K. [Multiple hepatic metastases due to germ cell tumor on initial clinical presentation]. *Nihon Kokyuki Gakkai Zasshi* 2007; **45**: 318-323 [PMID: 17491309 DOI: 10.2169/internalmedicine.46.6205]
- 9 Sosnoff DR, Friend RB, Berkovic M, Rasansky RJ, Hoffman SM. Salivary amylase-producing multiple myeloma: case report and review of the current literature. *J Clin Oncol* 2013; **31**: e309-e311 [PMID: 23690421 DOI: 10.1200/JCO.2012.46.4677]
- 10 Domschke W, Tymnner F, Domschke S, Demling L. Exocrine pancreatic function in juvenile diabetics. *Am J Dig Dis* 1975; **20**: 309-312 [PMID: 1130359 DOI: 10.1007/BF01237787]
- 11 Frier BM, Saunders JH, Wormsley KG, Bouchier IA. Exocrine pancreatic function in juvenile-onset diabetes mellitus. *Gut* 1976; **17**: 685-691 [PMID: 976808 DOI: 10.1136/gut.17.9.685]
- 12 Frier BM, Faber OK, Binder C, Elliot HL. The effect of residual insulin secretion on exocrine pancreatic function in juvenile-onset diabetes mellitus. *Diabetologia* 1978; **14**: 301-304 [PMID: 348540 DOI: 10.1007/BF01223020]
- 13 Yokoyama J, Tajima N, Ikeda Y, Ohno M, Saito S, Sakamoto Y, Tanese T, Abe M. The amylase activity and its isoenzyme analysis in juvenile-onset diabetes mellitus. *Tonyobyoby* 1980; **23**: 607-617 [DOI: 10.11213/tonyobyoby1958.23.607]
- 14 Dandona P, Freedman DB, Foo Y, Perkins J, Katrak A, Mikhailidis DP, Rosalki SB, Beckett AG. Exocrine pancreatic function in diabetes mellitus. *J Clin Pathol* 1984; **37**: 302-306 [PMID: 6699193 DOI: 10.1136/jcp.37.3.302]
- 15 Swislocki A, Noth R, Hallstone A, Kyger E, Triadafilopoulos G. Secretin-stimulated amylase release into blood is impaired in type 1 diabetes mellitus. *Horm Metab Res* 2005; **37**: 326-330 [PMID: 15971157 DOI: 10.1055/s-2005-861478]
- 16 Ogawa Z, Hasegawa A. Amylase. *Rinsho Byori* 2001; **Suppl 116**: 36-44 [PMID: 11797378]
- 17 Skrha J, Stěpán J. Clinical significance of amylase isoenzyme determination. *Acta Univ Carol Med Monogr* 1987; **120**: 1-81 [PMID: 2446482]
- 18 Gillard BK, Cox KL, Pollack PA, Geffner ME. Cystic fibrosis serum pancreatic amylase. Useful discriminator of exocrine function. *Am J Dis Child* 1984; **138**: 577-580 [PMID: 6202136]
- 19 Wolf RO, Hubbard VS, Gillard BK, Kingman A. Three methods compared for determination of pancreatic and salivary amylase activity in serum of cystic fibrosis patients. *Clin Chem* 1986; **32**: 296-300 [PMID: 2417751]
- 20 Knight AH, Williams DN, Ellis G, Goldberg DM. Significance of hyperamylasaemia and abdominal pain in diabetic ketoacidosis. *Br Med J* 1973; **3**: 128-131 [PMID: 4198367 DOI: 10.1136/bmj.3.5872.128]
- 21 Yadav D, Nair S, Norkus EP, Pitchumoni CS. Nonspecific hyperamylasemia and hyperlipasemia in diabetic ketoacidosis: incidence and correlation with biochemical abnormalities. *Am J Gastroenterol* 2000; **95**: 3123-3128 [PMID: 11095328 DOI: 10.1111/j.1572-0241.2000.03279.x]
- 22 Rizvi AA. Serum amylase and lipase in diabetic ketoacidosis. *Diabetes Care* 2003; **26**: 3193-3194 [PMID: 14578269]
- 23 Somogyi M. Blood diastase in health and diabetes. *J Biol Chem* 1940; **134**: 315-318
- 24 Quintanilla-Flores DL, Rendón-Ramírez EJ, Colunga-Pedraza PR, Gallardo-Escamilla J, Corral-Benavides SA, González-González JG, Tamez-Pérez HE. Clinical course of diabetic ketoacidosis in hypertriglyceridemic pancreatitis. *Pancreas* 2015; **44**: 615-618 [PMID: 25785723 DOI: 10.1097/MPA.0000000000000300]
- 25 Korc M, Owerbach D, Quinto C, Rutter WJ. Pancreatic islet-acinar cell interaction: amylase messenger RNA levels are determined by insulin. *Science* 1981; **213**: 351-353 [PMID: 6166044 DOI: 10.1126/science.6166044]
- 26 Mössner J, Logsdon CD, Williams JA, Goldfine ID. Insulin, via its own receptor, regulates growth and amylase synthesis in pancreatic acinar AR42J cells. *Diabetes* 1985; **34**: 891-897 [PMID: 2411617]
- 27 Nater UM, La Marca R, Florin L, Moses A, Langhans W, Koller MM, Ehlert U. Stress-induced changes in human salivary alpha-amylase activity -- associations with adrenergic activity. *Psychoneuroendocrinology* 2006; **31**: 49-58 [PMID: 16002223 DOI: 10.1016/j.psyneuen.2005.05.010]
- 28 Schumacher S, Kirschbaum C, Fydrich T, Ströhle A. Is salivary alpha-amylase an indicator of autonomic nervous system dysregulations in mental disorders?--a review of preliminary findings and the interactions with cortisol. *Psychoneuroendocrinology* 2013; **38**: 729-743 [PMID: 23481259 DOI: 10.1016/j.psyneuen.2013.02.003]
- 29 Etxeberria U, de la Garza AL, Campión J, Martínez JA, Milagro FI. Antidiabetic effects of natural plant extracts via inhibition of carbohydrate hydrolysis enzymes with emphasis on pancreatic alpha amylase. *Expert Opin Ther Targets* 2012; **16**: 269-297 [PMID: 22360606 DOI: 10.1517/14728222.2012.664134]
- 30 Barrett ML, Udani JK. A proprietary alpha-amylase inhibitor from white bean (*Phaseolus vulgaris*): a review of clinical studies on weight loss and glycemic control. *Nutr J* 2011; **10**: 24 [PMID: 21414227 DOI: 10.1186/1475-2891-10-24]
- 31 Scannapieco FA, Torres G, Levine MJ. Salivary alpha-amylase: role in dental plaque and caries formation. *Crit Rev Oral Biol Med* 1993; **4**: 301-307 [PMID: 8373987]
- 32 Sánchez GA, Miozza VA, Delgado A, Busch L. Relationship between salivary mucin or amylase and the periodontal status. *Oral Dis* 2013; **19**: 585-591 [PMID: 23170808 DOI: 10.1111/odi.12039]
- 33 Carter DA, Wobken JD, Dixit PK, Bauer GE. Immunoreactive insulin in rat salivary glands and its dependence on age and serum insulin levels. *Proc Soc Exp Biol Med* 1995; **209**: 245-250 [PMID: 7777586 DOI: 10.3181/00379727-209-43899]
- 34 Rocha EM, Carvalho CR, Saad MJ, Velloso LA. The influence of ageing on the insulin signalling system in rat lacrimal and salivary glands. *Acta Ophthalmol Scand* 2003; **81**: 639-645 [PMID: 14641268 DOI: 10.1111/j.1395-3907.2003.00162.x]
- 35 Schneeman BO, Inman MD, Stern JS. Pancreatic enzyme activity

- in obese and lean Zucker rats: a developmental study. *J Nutr* 1983; **113**: 921-925 [PMID: 6187904]
- 36 **Kondo T**, Hayakawa T, Shibata T, Sato Y, Toda Y. Serum levels of pancreatic enzymes in lean and obese subjects. *Int J Pancreatol* 1988; **3**: 241-248 [PMID: 2455007]
- 37 **de Oliveira CG**, Collares EF, Barbieri MA, Fernandes MI. Production and concentration of saliva and salivary amylase in obese children. *Arq Gastroenterol* 1997; **34**: 105-111 [PMID: 9496426]
- 38 **Nakajima K**, Nemoto T, Muneyuki T, Kakei M, Fuchigami H, Munakata H. Low serum amylase in association with metabolic syndrome and diabetes: A community-based study. *Cardiovasc Diabetol* 2011; **10**: 34 [PMID: 21496338 DOI: 10.1186/1475-2840-10-34]
- 39 **Tarantino G**, Finelli C. What about non-alcoholic fatty liver disease as a new criterion to define metabolic syndrome? *World J Gastroenterol* 2013; **19**: 3375-3384 [PMID: 23801829 DOI: 10.3748/wjg.v19.i22.3375]
- 40 **Nakajima K**, Oshida H, Muneyuki T, Saito M, Hori Y, Fuchigami H, Kakei M, Munakata H. Independent association between low serum amylase and non-alcoholic fatty liver disease in asymptomatic adults: a cross-sectional observational study. *BMJ Open* 2013; **3**: [PMID: 23293250 DOI: 10.1136/bmjopen-2012-002235]
- 41 **Lee JG**, Park SW, Cho BM, Lee S, Kim YJ, Jeong DW, Yi YH, Cho YH. Serum amylase and risk of the metabolic syndrome in Korean adults. *Clin Chim Acta* 2011; **412**: 1848-1853 [PMID: 21726545 DOI: 10.1016/j.cca.2011.06.023]
- 42 **Zhao Y**, Zhang J, Zhang J, Wu J, Chen Y. Metabolic syndrome and diabetes are associated with low serum amylase in a Chinese asymptomatic population. *Scand J Clin Lab Invest* 2014; **74**: 235-239 [PMID: 24456421 DOI: 10.3109/00365513.2013.878469]
- 43 **Yao J**, Zhao Y, Zhang J, Hong Y, Lu H, Wu J. Serum amylase levels are decreased in Chinese non-alcoholic fatty liver disease patients. *Lipids Health Dis* 2014; **13**: 185 [PMID: 25481429 DOI: 10.1186/1476-511X-13-185]
- 44 **Muneyuki T**, Nakajima K, Aoki A, Yoshida M, Fuchigami H, Munakata H, Ishikawa SE, Sugawara H, Kawakami M, Momomura S, Kakei M. Latent associations of low serum amylase with decreased plasma insulin levels and insulin resistance in asymptomatic middle-aged adults. *Cardiovasc Diabetol* 2012; **11**: 80 [PMID: 22748134 DOI: 10.1186/1475-2840-11-80]
- 45 **Curd R**, Crook MA. Causes of hypoamylasaemia in a hospital population. *Scand J Clin Lab Invest* 2015; **75**: 585-587 [PMID: 26203959 DOI: 10.3109/00365513.2015.1060520]
- 46 **Williams JA**, Goldfine ID. The insulin-pancreatic acinar axis. *Diabetes* 1985; **34**: 980-986 [PMID: 2412919]
- 47 **Nakajima K**, Muneyuki T, Munakata H, Kakei M. Revisiting the cardiometabolic relevance of serum amylase. *BMC Res Notes* 2011; **4**: 419 [PMID: 22004561 DOI: 10.1186/1756-0500-4-419]
- 48 **Blaak EE**, Antoine JM, Benton D, Björck I, Bozzetto L, Brouns F, Diamant M, Dye L, Hulshof T, Holst JJ, Lampert DJ, Laville M, Lawton CL, Meheust A, Nilsson A, Normand S, Rivellese AA, Theis S, Torekov SS, Vinoy S. Impact of postprandial glycaemia on health and prevention of disease. *Obes Rev* 2012; **13**: 923-984 [PMID: 22780564 DOI: 10.1111/j.1467-789X.2012.01011.x]
- 49 **Sandqvist M**, Strindberg L, Schmelz M, Lönnroth P, Jansson PA. Impaired delivery of insulin to adipose tissue and skeletal muscle in obese women with postprandial hyperglycemia. *J Clin Endocrinol Metab* 2011; **96**: E1320-E1324 [PMID: 21677042 DOI: 10.1210/jc.2011-0233]
- 50 **Boehlke C**, Zierau O, Hannig C. Salivary amylase - The enzyme of unspecialized euryphagous animals. *Arch Oral Biol* 2015; **60**: 1162-1176 [PMID: 26043446 DOI: 10.1016/j.archoralbio.2015.05.008]
- 51 **Mandel AL**, Peyrot des Gachons C, Plank KL, Alarcon S, Breslin PA. Individual differences in AMY1 gene copy number, salivary  $\alpha$ -amylase levels, and the perception of oral starch. *PLoS One* 2010; **5**: e13352 [PMID: 20967220 DOI: 10.1371/journal.pone.0013352]
- 52 **Mandel AL**, Breslin PA. High endogenous salivary amylase activity is associated with improved glycemic homeostasis following starch ingestion in adults. *J Nutr* 2012; **142**: 853-858 [PMID: 22492122 DOI: 10.3945/jn.111.156984]
- 53 **Tye JG**, Karn RC, Merritt AD. Differential expression of salivary (Amy1) and pancreatic (Amy2) human amylase loci in prenatal and postnatal development. *J Med Genet* 1976; **13**: 96-102 [PMID: 933119 DOI: 10.1136/jmg.13.2.96]
- 54 **Davis MM**, Hodes ME, Munsick RA, Ulbright TM, Goldstein DJ. Pancreatic amylase expression in human pancreatic development. *Hybridoma* 1986; **5**: 137-145 [PMID: 2424823 DOI: 10.1089/hyb.1986.5.137]
- 55 **Falchi M**, El-Sayed Moustafa JS, Takousis P, Pesce F, Bonnefond A, Andersson-Assarsson JC, Sudmant PH, Dorajoo R, Al-Shafai MN, Bottolo L, Ozdemir E, So HC, Davies RW, Patrice A, Dent R, Mangino M, Hysi PG, Dechaume A, Huyvaert M, Skinner J, Pigeyre M, Caiazzo R, Raverdy V, Vaillant E, Field S, Balkau B, Marre M, Visvikis-Siest S, Weill J, Poulain-Godefroy O, Jacobson P, Sjöström L, Hammond CJ, Deloukas P, Sham PC, McPherson R, Lee J, Tai ES, Sladek R, Carlsson LM, Walley A, Eichler EE, Pattou F, Spector TD, Froguel P. Low copy number of the salivary amylase gene predisposes to obesity. *Nat Genet* 2014; **46**: 492-497 [PMID: 24686848 DOI: 10.1038/ng.2939]
- 56 **Choi YJ**, Nam YS, Yun JM, Park JH, Cho BL, Son HY, Kim JI, Yun JW. Association between salivary amylase (AMY1) gene copy numbers and insulin resistance in asymptomatic Korean men. *Diabet Med* 2015; **32**: 1588-1595 [PMID: 25996848 DOI: 10.1111/dme.12808]
- 57 **Carpenter D**, Dhar S, Mitchell LM, Fu B, Tyson J, Shwan NA, Yang F, Thomas MG, Armour JA. Obesity, starch digestion and amylase: association between copy number variants at human salivary (AMY1) and pancreatic (AMY2) amylase genes. *Hum Mol Genet* 2015; **24**: 3472-3480 [PMID: 25788522 DOI: 10.1093/hmg/ddv098]
- 58 **Perry GH**, Dominy NJ, Claw KG, Lee AS, Fiegler H, Redon R, Werner J, Villanea FA, Mountain JL, Misra R, Carter NP, Lee C, Stone AC. Diet and the evolution of human amylase gene copy number variation. *Nat Genet* 2007; **39**: 1256-1260 [PMID: 17828263 DOI: 10.1038/ng2123]
- 59 **Usher CL**, Handsaker RE, Esko T, Tuke MA, Weedon MN, Hastie AR, Cao H, Moon JE, Kashin S, Fuchsberger C, Metspalu A, Pato CN, Pato MT, McCarthy MI, Boehnke M, Altshuler DM, Frayling TM, Hirschhorn JN, McCarroll SA. Structural forms of the human amylase locus and their relationships to SNPs, haplotypes and obesity. *Nat Genet* 2015; **47**: 921-925 [PMID: 26098870 DOI: 10.1038/ng.3340]
- 60 **Greenhill C**. Obesity. Copy number variants in AMY1 connected with obesity via carbohydrate metabolism. *Nat Rev Endocrinol* 2014; **10**: 312 [PMID: 24732973 DOI: 10.1038/nrendo.2014.54]
- 61 **Mejía-Benítez MA**, Bonnefond A, Yengo L, Huyvaert M, Dechaume A, Peralta-Romero J, Klünder-Klünder M, García Mena J, El-Sayed Moustafa JS, Falchi M, Cruz M, Froguel P. Beneficial effect of a high number of copies of salivary amylase AMY1 gene on obesity risk in Mexican children. *Diabetologia* 2015; **58**: 290-294 [PMID: 25394825 DOI: 10.1007/s00125-014-3441-3]
- 62 **Viljakainen H**, Andersson-Assarsson JC, Armenio M, Pekkinen M, Pettersson M, Valtä H, Lipsanen-Nyman M, Mäkitie O, Lindstrand A. Low Copy Number of the AMY1 Locus Is Associated with Early-Onset Female Obesity in Finland. *PLoS One* 2015; **10**: e0131883 [PMID: 26132294 DOI: 10.1371/journal.pone.0131883]
- 63 **Arendt M**, Fall T, Lindblad-Toh K, Axelsson E. Amylase activity is associated with AMY2B copy numbers in dog: implications for dog domestication, diet and diabetes. *Anim Genet* 2014; **45**: 716-722 [PMID: 24975239 DOI: 10.1111/age.12179]
- 64 **Maruyama K**, Takahashi H, Okuyama K, Yokoyama A, Nakamura Y, Kobayashi Y, Ishii H. Low serum amylase levels in drinking alcoholics. *Alcohol Clin Exp Res* 2003; **27**: 16S-21S [PMID: 12960501 DOI: 10.1097/01.ALC.0000078827.46112.76]



- 65 **Li J**, Zhou C, Wang R, Liu R, Huang Z, Tang C. Irreversible exocrine pancreatic insufficiency in alcoholic rats without chronic pancreatitis after alcohol withdrawal. *Alcohol Clin Exp Res* 2010; **34**: 1843-1848 [PMID: 20662806 DOI: 10.1111/j.1530-0277.2010.01272.x]
- 66 **Enberg N**, Alho H, Loimaranta V, Lenander-Lumikari M. Saliva flow rate, amylase activity, and protein and electrolyte concentrations in saliva after acute alcohol consumption. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2001; **92**: 292-298 [PMID: 11552146 DOI: 10.1067/moe.2001.116814]
- 67 **Schrieks IC**, Heil AL, Hendriks HF, Mukamal KJ, Beulens JW. The effect of alcohol consumption on insulin sensitivity and glycemic status: a systematic review and meta-analysis of intervention studies. *Diabetes Care* 2015; **38**: 723-732 [PMID: 25805864 DOI: 10.2337/dc14-1556]
- 68 **Poli A**, Marangoni F, Avogaro A, Barba G, Bellentani S, Bucci M, Cambieri R, Catapano AL, Costanzo S, Cricelli C, de Gaetano G, Di Castelnuovo A, Faggiano P, Fattorioli F, Fontana L, Forlani G, Frattini S, Giacco R, La Vecchia C, Lazzaretto L, Loffredo L, Lucchin L, Marelli G, Marrocco W, Minisola S, Musico M, Novo S, Nazzoli C, Pelucchi C, Perri L, Pieralli F, Rizzoni D, Sterzi R, Vettor R, Violi F, Visioli F. Moderate alcohol use and health: a consensus document. *Nutr Metab Cardiovasc Dis* 2013; **23**: 487-504 [PMID: 23642930 DOI: 10.1016/j.numecd.2013.02.007]
- 69 **Callegari C**, Lami F. Cigarette smoking and salivary amylase activity. *Gut* 1984; **25**: 909 [PMID: 6611285 DOI: 10.1136/gut.25.8.909]
- 70 **Nourane YA**, Alaa D. Effect of Smoking on Serum Amylase and Lipase Enzymes. *J Am Sci* 2012; **8**: 406-410
- 71 **Oshida H**, Kutsuma A, Nakajima K. Associations of eating a late-evening meal before bedtime with low serum amylase and unhealthy conditions. *J Diabetes Metab Disord* 2013; **12**: 53 [PMID: 24354901 DOI: 10.1186/2251-6581-12-53]
- 72 **Koibuchi E**, Suzuki Y. Exercise upregulates salivary amylase in humans (Review). *Exp Ther Med* 2014; **7**: 773-777 [PMID: 24669232]
- 73 **Lippi G**, Salvagno GL, Danese E, Tarperi C, La Torre A, Guidi GC, Schena F. The baseline serum value of  $\alpha$ -amylase is a significant predictor of distance running performance. *Clin Chem Lab Med* 2015; **53**: 469-476 [PMID: 25274961 DOI: 10.1515/cclm-2014-0850]
- 74 **Nakajima K**. High serum amylase levels may reflect a wide spectrum of health benefits. *Clin Chem Lab Med* 2015; **53**: e67-e68 [PMID: 25490033 DOI: 10.1515/cclm-2014-1120]
- 75 **Ozkok A**, Elcioglu OC, Cukadar T, Bakan A, Sasak G, Atilgan KG, Alisir S, Kanbay M, Covic A, Odabas AR. Low serum pancreatic enzyme levels predict mortality and are associated with malnutrition-inflammation-atherosclerosis syndrome in patients with chronic kidney disease. *Int Urol Nephrol* 2013; **45**: 477-484 [PMID: 22907629 DOI: 10.1007/s11255-012-0237-6]
- 76 **Shimizu Y**, Ichihara K. Sources of variation analysis and derivation of reference intervals for ALP, LDH, and amylase isozymes using sera from the Asian multicenter study on reference values. *Clin Chim Acta* 2015; **446**: 64-72 [PMID: 25843264 DOI: 10.1016/j.cca.2015.03.034]
- 77 **Nakajima K**, Oda E. Lower serum amylase in A blood type relative to O blood type in a general Japanese adult population. *Clin Chim Acta* 2015; **450**: 181-183 [PMID: 26301747 DOI: 10.1016/j.cca.2015.08.016]
- 78 **Qureshi MA**, Bhatti R. Frequency of ABO blood groups among the diabetes mellitus type 2 patients. *J Coll Physicians Surg Pak* 2003; **13**: 453-455 [PMID: 12921683]
- 79 **Fagherazzi G**, Gusto G, Clavel-Chapelon F, Balkau B, Bonnet F. ABO and Rhesus blood groups and risk of type 2 diabetes: evidence from the large E3N cohort study. *Diabetologia* 2015; **58**: 519-522 [PMID: 25533388 DOI: 10.1007/s00125-014-3472-9]
- 80 **Amundadottir L**, Kraft P, Stolzenberg-Solomon RZ, Fuchs CS, Petersen GM, Arslan AA, Bueno-de-Mesquita HB, Gross M, Helzlsouer K, Jacobs EJ, LaCroix A, Zheng W, Albanes D, Bamlet W, Berg CD, Berrino F, Bingham S, Buring JE, Bracci PM, Canzian F, Clavel-Chapelon F, Clipp S, Cotterchio M, de Andrade M, Duell EJ, Fox JW, Gallinger S, Gaziano JM, Giovannucci EL, Goggins M, González CA, Hallmans G, Hankinson SE, Hassan M, Holly EA, Hunter DJ, Hutchinson A, Jackson R, Jacobs KB, Jenab M, Kaaks R, Klein AP, Kooperberg C, Kurtz RC, Li D, Lynch SM, Mandelsson M, McWilliams RR, Mendelsohn JB, Michaud DS, Olson SH, Overvad K, Patel AV, Peeters PH, Rajkovic A, Riboli E, Risch HA, Shu XO, Thomas G, Tobias GS, Trichopoulos D, Van Den Eeden SK, Virtamo J, Wactawski-Wende J, Wolpin BM, Yu H, Yu K, Zeleniuch-Jacquotte A, Chanock SJ, Hartge P, Hoover RN. Genome-wide association study identifies variants in the ABO locus associated with susceptibility to pancreatic cancer. *Nat Genet* 2009; **41**: 986-990 [PMID: 19648918 DOI: 10.1038/ng.429]
- 81 **Iodice S**, Maisonneuve P, Botteri E, Sandri MT, Lowenfels AB. ABO blood group and cancer. *Eur J Cancer* 2010; **46**: 3345-3350 [PMID: 20833034 DOI: 10.1016/j.ejca.2010.08.009]
- 82 **Nakao M**, Matsuo K, Hosono S, Ogata S, Ito H, Watanabe M, Mizuno N, Iida S, Sato S, Yatabe Y, Yamao K, Ueda R, Tajima K, Tanaka H. ABO blood group alleles and the risk of pancreatic cancer in a Japanese population. *Cancer Sci* 2011; **102**: 1076-1080 [PMID: 21306478 DOI: 10.1111/j.1349-7006.2011.01907.x]
- 83 **Kaye WH**, Gendall K, Kye C. The role of the central nervous system in the psychoneuroendocrine disturbances of anorexia and bulimia nervosa. *Psychiatr Clin North Am* 1998; **21**: 381-396 [PMID: 9670232 DOI: 10.1016/S0193-953X(05)70011-3]
- 84 **Mehler PS**, Krantz MJ, Sachs KV. Treatments of medical complications of anorexia nervosa and bulimia nervosa. *J Eat Disord* 2015; **3**: 15 [PMID: 25874112 DOI: 10.1186/s40337-015-0041-7]
- 85 **Humphries LL**, Adams LJ, Eckfeldt JH, Levitt MD, McClain CJ. Hyperamylasemia in patients with eating disorders. *Ann Intern Med* 1987; **106**: 50-52 [PMID: 2431640 DOI: 10.7326/0003-4819-106-1-50]
- 86 **Robertson C**, Millar H. Hyperamylasemia in bulimia nervosa and hyperemesis gravidarum. *Int J Eat Disord* 1999; **26**: 223-227 [PMID: 10422613 DOI: 10.1002/(SICI)1098-108X(199909)26]
- 87 **Wolfe BE**, Jimerson DC, Smith A, Keel PK. Serum amylase in bulimia nervosa and purging disorder: differentiating the association with binge eating versus purging behavior. *Physiol Behav* 2011; **104**: 684-686 [PMID: 21781981 DOI: 10.1016/j.physbeh.2011.06.025]
- 88 **Collen MJ**, Ansher AF, Chapman AB, Mackow RC, Lewis JH. Serum amylase in patients with renal insufficiency and renal failure. *Am J Gastroenterol* 1990; **85**: 1377-1380 [PMID: 1699413]
- 89 **Kurt Ö**, Demirci H, Ozturk K, Kantarcioglu M, Uygur A. Severe serum amylase elevation, with only chronic kidney disease. *Ren Fail* 2015; **37**: 915 [PMID: 25774630 DOI: 10.3109/0886022X.2015.1022852]
- 90 **Lando HM**, Alattar M, Dua AP. Elevated amylase and lipase levels in patients using glucagonlike peptide-1 receptor agonists or dipeptidyl-peptidase-4 inhibitors in the outpatient setting. *Endocr Pract* 2012; **18**: 472-477 [PMID: 22440997 DOI: 10.4158/EP11290.OR]
- 91 **Tokuyama H**, Kawamura H, Fujimoto M, Kobayashi K, Nieda M, Okazawa T, Takemoto M, Shimada F. A low-grade increase of serum pancreatic exocrine enzyme levels by dipeptidyl peptidase-4 inhibitor in patients with type 2 diabetes. *Diabetes Res Clin Pract* 2013; **100**: e66-e69 [PMID: 23618553 DOI: 10.1016/j.diabetes.2013.03.034]
- 92 **Lengyel Z**. Report all increases in serum amylase in patients starting incretins. *BMJ* 2013; **347**: f5333 [PMID: 24009264 DOI: 10.1136/bmj.f5333]
- 93 **Layzer P**, Rizza RA, Zinsmeister AR, Carlson GL, DiMaggio EP. Effect of a purified amylase inhibitor on carbohydrate tolerance in normal subjects and patients with diabetes mellitus. *Mayo Clin Proc* 1986; **61**: 442-447 [PMID: 2423813 DOI: 10.1016/S0025-6196(12)61978-8]
- 94 **Van Gaal L**, Mertens I, Ballaux D, Verkade HJ. Modern, new pharmacotherapy for obesity. A gastrointestinal approach. *Best Pract Res Clin Gastroenterol* 2004; **18**: 1049-1072 [PMID:

15561638 DOI: 10.1016/j.bpg.2004.09.001]

- 95 **Tucci SA**, Boyland EJ, Halford JC. The role of lipid and carbohydrate digestive enzyme inhibitors in the management of

obesity: a review of current and emerging therapeutic agents. *Diabetes Metab Syndr Obes* 2010; **3**: 125-143 [PMID: 21437083 DOI: 10.2147/DMSOTT.S7005]

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## Metformin revisited: Does this regulator of AMP-activated protein kinase secondarily affect bone metabolism and prevent diabetic osteopathy?

Antonio Desmond McCarthy, Ana María Cortizo, Claudia Sedlinsky

Antonio Desmond McCarthy, Ana María Cortizo, Claudia Sedlinsky, Laboratorio de Investigaciones en Osteopatías y Metabolismo Mineral, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata 1900, Argentina

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**Correspondence to:** Antonio Desmond McCarthy, Professor, Laboratorio de Investigaciones en Osteopatías y Metabolismo Mineral, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Calle 50 y 115, La Plata 1900, Argentina. [mccarthy@biol.unlp.edu.ar](mailto:mccarthy@biol.unlp.edu.ar)  
Telephone: +54-221-4512426  
Fax: +54-221-4512426

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### Abstract

Patients with long-term type 1 and type 2 diabetes

mellitus (DM) can develop skeletal complications or "diabetic osteopathy". These include osteopenia, osteoporosis and an increased incidence of low-stress fractures. In this context, it is important to evaluate whether current anti-diabetic treatments can secondarily affect bone metabolism. Adenosine monophosphate-activated protein kinase (AMPK) modulates multiple metabolic pathways and acts as a sensor of the cellular energy status; recent evidence suggests a critical role for AMPK in bone homeostasis. In addition, AMPK activation is believed to mediate most clinical effects of the insulin-sensitizer metformin. Over the past decade, several research groups have investigated the effects of metformin on bone, providing a considerable body of pre-clinical (*in vitro*, *ex vivo* and *in vivo*) as well as clinical evidence for an anabolic action of metformin on bone. However, two caveats should be kept in mind when considering metformin treatment for a patient with type 2 DM at risk for diabetic osteopathy. In the first place, metformin should probably not be considered an anti-osteoporotic drug; it is an insulin sensitizer with proven macrovascular benefits that can secondarily improve bone metabolism in the context of DM. Secondly, we are still awaiting the results of randomized placebo-controlled studies in humans that evaluate the effects of metformin on bone metabolism as a primary endpoint.

**Key words:** Diabetes mellitus; Osteoporosis; Bone fractures; Metformin; AMP-activated kinase

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**Core tip:** Patients with long-term type 1 and type 2 diabetes mellitus (DM) can develop skeletal complications. These include osteopenia, osteoporosis and increased incidence of low-stress fractures. In this context, it is important to evaluate whether current anti-diabetic treatments can secondarily affect bone metabolism. Over the past decade, several research

groups have investigated the effects of metformin on bone, providing a considerable body of pre-clinical (*in vitro*, *ex vivo* and *in vivo*) as well as clinical evidence for an anabolic action of metformin on bone. This could be particularly relevant when considering treatment options for DM in the context of diabetic osteopathy.

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## INTRODUCTION

Diabetes mellitus (DM) is a highly prevalent global disease associated with long-term microvascular and macrovascular complications. Over the past 30 years, an increasing body of experimental and clinical evidence has reported the association of type 1 and type 2 DM with osteopenia, osteoporosis and an increased incidence of low-stress fractures, in what has been called diabetic osteopathy<sup>[1]</sup>. Many adult patients with type 1 DM show mild osteopenia, with a decrease in bone mineral density (BMD) of around 10%<sup>[2]</sup> that would be expected to double the risk of non-vertebral fragility fractures<sup>[3]</sup>. However, the incidence of low-stress fractures in type 1 DM is 7-12 times that of age-matched non-diabetic individuals<sup>[4,5]</sup>. On the other hand, patients with type 2 DM tend to have normal or even moderately elevated BMD. Although this would be expected to reduce their incidence of osteoporotic fractures, they actually show a 2-fold increase in hip, extremity and vertebral fractures<sup>[3-7]</sup>. Taken together, these clinical observations are considered to be evidence for a significant decrease in bone quality of patients with both types of DM<sup>[8]</sup> that would explain their increase in low-stress fractures.

Several mechanisms have been proposed to explain diabetic osteopathy, such as disturbed glucose metabolism, tissue (bone) and systemic low-grade inflammation, changes in the secretory pattern of growth factors and/or cytokines, increased oxidative stress and excess accumulation of advanced glycation end products (AGEs). In particular, excess accumulation of AGEs in bone extracellular matrix (ECM) occurs as a function of aging and duration of Diabetes, and has been found to impair the mechanical properties of bone<sup>[9]</sup>. Poorly compensated DM elevates circulating reactive oxygen species (ROS), glucose and/or carbonyl stress, which can induce excess AGEs formation on bone ECM, reducing bone strength and post-yield properties. Additionally, collagen-AGEs interact with the receptor for AGEs (RAGE) expressed by osteoblasts and osteoclasts, inhibiting their functionality and decreasing bone turnover. This induces an even greater accumulation of AGEs in bone that contributes to

diabetic osteopathy, and can increase fracture risk<sup>[1]</sup>.

Treatment of patients with DM can include either an absolute requirement for exogenous insulin (type 1 DM), or relative requirement of glucose-lowering medication such as insulin and/or oral drugs (type 2 DM). Oral glucose-lowering agents fall into different classes that include (but are not limited to) insulin secretagogues, insulin sensitizers, incretin-based treatments and inhibitors of renal proximal tubule glucose reabsorption. Each class operates through distinct biological pathways and has certain advantages as well as disadvantages. Recently, several commonly prescribed oral medications for type 2 DM have been found to secondarily affect bone metabolism, in some cases modifying the incidence of fragility fractures<sup>[10]</sup>. Depending on their specific skeletal effects these drugs could either help to prevent diabetic osteopathy, or contribute to worsen this complication of DM.

Members of the thiazolidinediones (TZD) family of insulin-sensitizers such as rosiglitazone have been shown to be detrimental for bone health. In rodents, TZD increase the adipocytic commitment of mesenchymal stem cells (MSC) while decreasing their osteogenic potential, *via* a decrease in the Runx2/PPAR $\gamma$  ratio. This increases bone marrow adiposity and promotes bone loss<sup>[11]</sup>. In the ADOPT clinical trial<sup>[12]</sup>, results showed a higher risk of fracture in diabetic women, but not men, on rosiglitazone monotherapy.

Post-prandial incretin secretion is believed to play a physiological role linking nutrient ingestion to suppression of bone resorption and stimulation of bone formation. Thus, incretin-based treatments would be expected to show anabolic effects on bone, as has been suggested in a recent meta-analysis<sup>[13]</sup>. However, this may not be so in certain cases such as the DPP4 inhibitor saxagliptin, which has been found to impair MSC osteogenic potential and bone micro-architecture in rodent models<sup>[14]</sup>.

Inhibitors of the sodium glucose cotransporter 2 such as dapagliflozin and canagliflozin, that decrease plasma glucose and body weight by impairing proximal tubule glucose reabsorption, have recently been associated with alterations in mineral metabolism and with an increase in bone fractures. This undesirable effect is probably due to the fact that these drugs can induce hyperphosphataemia and an increase in fibroblast growth factor-23 and para-thyroid hormone levels<sup>[15]</sup>.

Metformin is an insulin-sensitizing biguanide that was developed several decades ago; however, it is still the most widely used oral anti-diabetic medication, particularly since the United Kingdom Prospective Diabetes Study demonstrated its efficacy for reducing macrovascular complications in obese type 2 DM patients<sup>[16]</sup>. Although the exact mechanism of action for metformin is still incompletely understood, in various tissues and organs it improves glucose metabolism *via* activation of the ubiquitously expressed AMP-activated protein kinase (AMPK)<sup>[17,18]</sup>. AMPK subunit expression and activation is tissue-specific, with the  $\alpha$ 1 subunit



accounting for most of bone AMPK<sup>[19]</sup>. Over the last 10 years, pre-clinical and clinical evidence has accumulated pointing to an anabolic effect of metformin on bone, in part due to AMPK activation.

## AMPK-A KEY ENERGY SENSOR

AMPK is a Ser/Thr protein kinase that modulates multiple metabolic pathways and acts as a sensor of the cellular energy status<sup>[20]</sup>. AMPK is a heterotrimer of three subunits. The  $\alpha$  subunit holds the catalytic domain with a Ser/Thr kinase domain (KD). It contains a Thr172 residue, critical for the kinase activity. AMPK- $\alpha$  also contains a regulatory domain, which interacts with the KD of the unphosphorylated inactive form. The  $\beta$  subunit contains a carbohydrate-binding module and acts as a scaffold for the assembly of  $\alpha$  and  $\gamma$  subunits. It also defines the subcellular localization of AMPK as well as its substrate specificity. The  $\gamma$  subunit contains four cystathionine- $\beta$ -synthase (CBS) domains, which bind adenine nucleotides. Expression of all three subunits is required for AMPK activity. The mechanism of activation first requires the reversible phosphorylation of the  $\alpha$  subunit; then after AMP binding to the CBS domain of  $\gamma$  subunit, an allosteric stimulatory effect occurs. Several upstream kinases (AMPKK) are involved in the phosphorylation of AMPK, such as liver kinase B1 (LKB1) and Ca<sup>2+</sup>/calmodulin-dependent protein kinase kinase beta (CaMKK $\beta$ ). Activation of AMPK can occur by two mechanisms: One is mediated by an increase of AMP/ATP ratio (for instance during exercise), so this mechanism coordinates anabolic and catabolic pathways to equilibrate nutrient supply with energy expenditure<sup>[21]</sup>. Several compounds can activate AMPK by this mechanism: 5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR), H<sub>2</sub>O<sub>2</sub>, MAPK inhibitors, TZD, leptin, adiponectin and  $\alpha$ -lipoic acid<sup>[22]</sup>. In the second mechanism, AMPK activation can be independent of AMP/ATP ratio and involves alternative AMPK regulation. Agents such as peroxynitrite, metformin, estradiol, low glucose levels and several membrane receptor agonists can induce AMPK activation by this way.

It has recently been demonstrated that AMPK can participate in the control of whole-body energy homeostasis by integrating signals from diverse cellular environments<sup>[23]</sup>. AMPK participates in several physiological events, such as survival, growth and development. This kinase could also be implicated in pathological conditions such as type 2 DM, insulin resistance, cardiovascular disease and cancer. For instance, AMPK can control epigenetic processes in certain cells to avoid reproductive defects in their subsequent generations<sup>[24]</sup>; or it can suppress proinflammatory-signalling pathways in adipocytes<sup>[25]</sup>. It has recently been reported that in patients with insulin resistance, AMPK is depressed in adipose tissue; an effect that is associated with oxidative stress, increased expression of inflammatory cytokines and decreased expression of genes regulating oxidative

phosphorylation. On the contrary, AMPK activation increases mitochondrial biogenesis<sup>[26]</sup>. AMPK can also inhibit the proliferation of muscle stem cells, and induce the differentiation of endothelial cell progenitors<sup>[21]</sup>.

## ROLE OF AMPK IN CELL METABOLISM

AMPK functions as an intracellular sensor regulating energy balance in different cell types, and thus can regulate diverse metabolic pathways (Table 1).

In the liver, AMPK acts as a "metabolic master switch"<sup>[27]</sup>; its activation inhibits energy-consuming pathways and stimulates ATP-producing catabolic pathways. For instance, after AMPK activation in a fasting state, fatty acid synthesis is inhibited while mitochondrial oxidative phosphorylation is stimulated. These effects occur by a reduction in malonyl-CoA content that is mediated by inhibition of Acetyl-CoA carboxylase. In addition, malonyl CoA decarboxylase is activated, thus further increasing fatty acid oxidation. Activation of AMPK also suppresses glucose production (gluconeogenesis), as has been demonstrated in metformin-treated primary hepatocyte cultures<sup>[17]</sup>. This has been confirmed in mice with a liver-selective deletion of the *AMPK $\alpha$ 2* gene, which exhibit hyperglycaemia and glucose intolerance in the fasting state<sup>[27]</sup>.

In skeletal muscle, AMPK regulates energy expenditure during exercise in order to optimize and enhance energy production. It participates in the transition from more glycolytic fibres to more oxidative fibres, following exercise training<sup>[28]</sup>. A role for AMPK in the myocytic uptake and oxidation of fatty acids has also been postulated. In addition, during exercise an increase in ATP turnover is accompanied by enhanced glucose uptake, in turn associated with an increase in myocyte plasma membrane GLUT4 expression<sup>[21]</sup>. Thus, use of metformin in patients with type 2 DM will increase their AMPK-induced glucose uptake and disposal. AMPK activation has also been postulated to induce skeletal muscle regeneration, by regulating its post-injury inflammatory response<sup>[29]</sup>. Postnatal skeletal muscle regeneration involves stem cell reprogramming that induces their proliferation, differentiation and/or self-renovation, and activation of the AMPK pathway is believed to regulate these processes<sup>[21]</sup>. Due to the regulatory effects of AMPK on integrated metabolism, activation of AMPK is considered a therapeutic target for hyperglycaemic states. For instance metformin, an anti-diabetic drug that is widely used for treatment of patients with type 2 DM, suppresses hepatic glucose production and decreases plasma glucose levels *via* activation of AMPK pathways.

The precise effects of AMPK on bone metabolism are incompletely known; however, recent evidence supports an active role for this kinase in bone physiology<sup>[30]</sup>. Several reports have demonstrated that AMPK modulates bone cell differentiation and function. In AMPK $\alpha$ -deleted animals, a reduction was found in

**Table 1** Role of AMP-activated protein kinase activation on cell metabolism in different organs

Organ	Effect	Mechanism of action	Ref.
Liver	Inhibition of anabolic pathways	Inhibition of fatty acid synthesis Inhibition of gluconeogenesis	[27]
Skeletal muscle	Stimulation of ATP synthesis	Stimulation of Mitochondrial oxidative phosphorylation	[27]
	Regulation of energy expenditure during exercise	Favours the transition from glycolytic to oxidative skeletal muscle fibers	[28]
		Regulation of myocytic uptake and oxidation of fatty acids	[21]
		Enhanced glucose uptake <i>via</i> an increase in GLUT4 expression	[21]
Bone	Increase in skeletal muscle regeneration	Regulation of post-injury inflammatory response	[29]
	Increase in osteoblastogenesis	Stem cell reprogramming: Induction of proliferation, differentiation and self-renewal	[21]
		Increases MSC differentiation towards the osteoblastic lineage favouring Runx2 expression	[30,37]
		Decreases PPAR $\gamma$ expression diminishing MSC differentiation towards the adipocytic phenotype	[36,37]
	Decrease in osteoclastogenesis	Negative regulation of RANKL expression by osteoblasts	[35]

MSC: Mesenchymal stem cells; ATP: Adenosine triphosphate; GLUT4: Glucose transporter type 4; RANKL: Receptor activator for nuclear factor  $\kappa$ B ligand.

trabecular bone mass<sup>[31]</sup>. Single  $\alpha 1$  or  $\alpha 2$  knockout (KO) mice are viable, but the double KO is embryonically lethal<sup>[27]</sup>. In addition, histomorphometric analysis revealed that AMPK $\alpha 1$  KO mice show an elevated rate of bone remodelling *in vivo*, associated with increased osteoclastogenesis *in vitro*.

*In vitro* experiments have demonstrated that AMPK activation enhances osteogenesis<sup>[30,32]</sup> while compound C (an AMPK inhibitor) reduces osteoblastic mineralization<sup>[30]</sup>. In other experiments AICAR (an activator of AMPK) was found to stimulate alkaline phosphatase activity (ALP) and mineral nodule formation by rat calvaria-derived cells, while compound C suppressed these effects<sup>[33]</sup>. A decrease in AMPK activity has been reported during osteoblastic differentiation; this could be associated with the high-energy requirements of maturing osteoprogenitor cells<sup>[34]</sup>. Although studies of AMPK action on bone resorption have led to conflicting results, it appears that AMPK is a negative regulator for RANKL and can thus decrease osteoclast-mediated bone resorption<sup>[35]</sup>. The AMPK pathway may also be involved in regulating the fate of bone marrow stromal cells (MSC) toward the osteoblastic or adipocytic lineage by reciprocally regulating the expression of Runx2 and PPAR $\gamma$ <sup>[30]</sup>. AMPK has been shown on one hand to induce phosphorylation of  $\beta$ -catenin, suppress PPAR $\gamma$  expression and thus reduce adipogenesis<sup>[36]</sup>; while on the other hand it increases Runx2 expression and thus osteoblastic differentiation of MSC<sup>[37]</sup>. This evidence suggests a critical role for AMPK in bone homeostasis.

## MOLECULAR MECHANISMS OF METFORMIN ACTION

Metformin has been widely used in the United States since 1995 as an oral anti-diabetic treatment for type 2 DM<sup>[22]</sup>. Even though its precise mechanism of action is not completely known, metformin is known to activate AMPK<sup>[17]</sup>; however, AMPK-independent pathways have

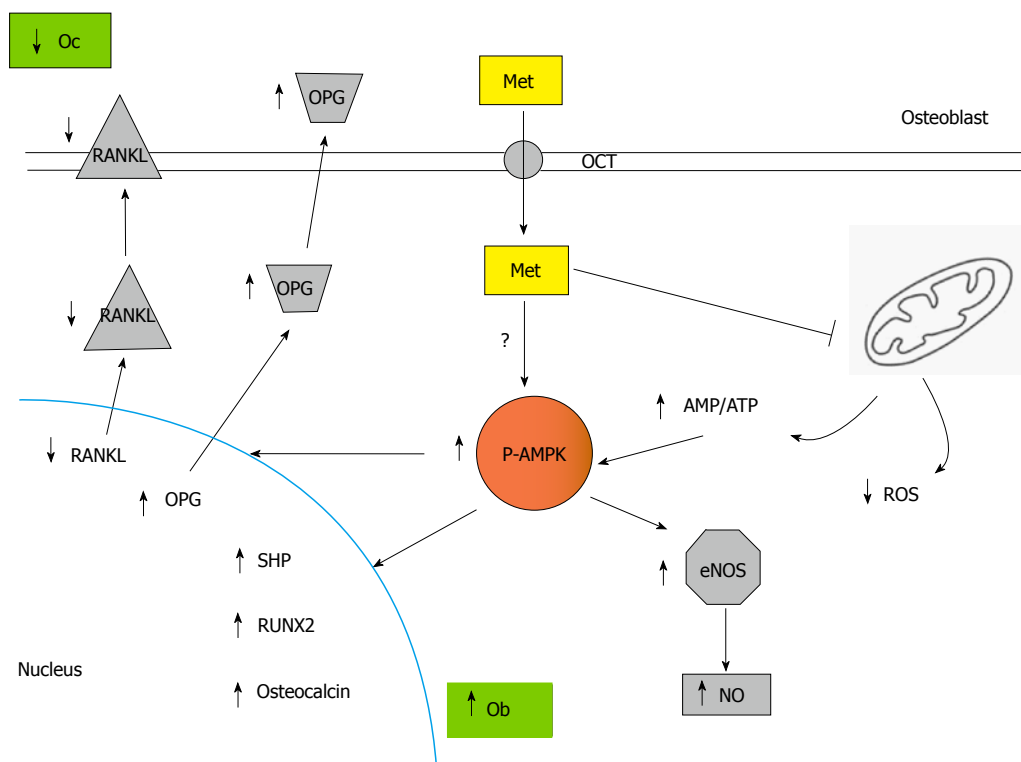
also been postulated<sup>[38]</sup>.

Metformin can be incorporated into cells by a facilitated mechanism that is mediated by different isoforms of the organic cation transporter. Metformin induces mild and specific inhibition of the mitochondrial respiratory chain complex in hepatocytes and other tissues<sup>[39]</sup>; it can also inhibit the mitochondrial production of ROS. Inhibition of mitochondrial activity induces a decrease in the cell energy status, which in turn triggers depletion of ATP and a diminished ATP/AMP ratio. This effect then induces phosphorylation and activation of AMPK *via* LKB1. Metformin also induces an acute inhibition of gluconeogenesis: this can be explained by the decrease in ATP/AMP ratio, which inhibits key enzymes of the gluconeogenic pathway such as fructose-1,6-bisphosphatase. New evidence suggests that this metformin-induced inhibition of glucose production could also be mediated by a down-regulation of gluconeogenic genes *via* a transcription-independent process. It has been suggested that reduction in energy status, but not AMPK activation, is critical for metformin inhibition of hepatic glucose production<sup>[38]</sup>.

In addition to its effects in the liver, metformin can also affect other organs *via* multiple molecular mechanisms. One important action of metformin is its reduction of endothelial activation and of atherogenesis<sup>[16]</sup>. Metformin decreases intracellular ROS production in endothelial cells by inhibiting both NADPH oxidation and the respiratory chain complex<sup>[40]</sup>, and this effect appears to be independent of AMPK activation.

## METFORMIN MEETS BONE

Over the past decade, several research groups have investigated the effects of metformin on bone. The results of these studies are discussed in detail below. They have provided a considerable body of pre-clinical (*in vitro*, *ex vivo* and *in vivo*) as well as clinical evidence for an anabolic action of metformin on bone, which could be particularly relevant when considering treatment



**Figure 1 Metformin actions via osteoblasts are pro-osteogenic and anti-resorptive.** Metformin is incorporated into osteoblasts, where it inhibits intracellular ROS production and induces AMPK phosphorylation/activation. This increases eNOS activity and NO production, promoting osteoblast proliferation. In addition, activated AMPK up regulates osteoblastic differentiation and mineralization via expression of Runx2 and SHP, while decreasing osteoclastic recruitment and bone-resorbing activity through a reduction in osteoblastic RANKL/OPG ratio. ROS: Reactive oxygen species; AMPK: AMP-activated protein kinase; OPG: Osteoprotegerin; RANKL: Reduced receptor activator of nuclear factor- $\kappa$ B ligand; SHP: Small heterodimer partner; RUNX2: Runt-related transcription factor 2; AMP: Adenosine monophosphate; ATP: Adenosine triphosphate; Met: Metformin.

options for DM in the context of diabetic osteopathy.

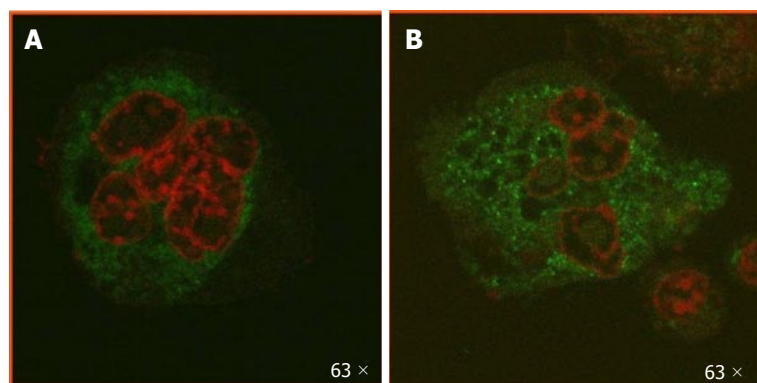
### ***In vitro* effects of metformin on bone cells**

Metformin has been found to modulate the physiology of osteoblasts (Figure 1) and osteoclasts, as well as influencing the phenotypic progression of bone MSC.

Cortizo *et al.*<sup>[41]</sup> were the first to describe an *in vitro* effect of metformin on bone-derived cells, showing that it dose-dependently increased osteoblastic proliferation, differentiation and mineralization. This effect was mediated by activation of extracellular-regulated kinases and by induction of NO synthases. Several researchers have corroborated these results<sup>[19,42-44]</sup>, additionally showing that the osteogenic *in vitro* action of metformin on osteoblasts is dependent on activation of the AMPK signalling pathway and subsequent bone morphogenetic protein-2 production. In an interesting mechanistic study, Jang *et al.*<sup>[45]</sup> found that metformin increased the osteoblastic transcription of small heterodimer partner (SHP) and osteocalcin genes, an effect that was inhibited either by a dominant negative form of AMPK or by compound C. They also found that metformin-induced SHP gene expression was mediated by upstream stimulatory factor-1 (USF-1), that AMPK activation increased the expression of Runx2 and that SHP interacts physically and forms a complex with Runx2 on the osteocalcin gene promoter in osteoblastic cells. Thus, metformin appears to enhance osteoblast

differentiation through the transactivation of Runx2 via the AMPK/USF-1/SHP regulatory cascade<sup>[45]</sup>. In another study, Mai *et al.*<sup>[46]</sup> found that metformin dose-dependently stimulated osteoprotegerin (OPG) and reduced receptor activator of nuclear factor- $\kappa$ B ligand (RANKL) mRNA and protein expression by cultured osteoblastic cells, a potentially anti-osteoclastogenic effect that they were able to block by inhibition of AMPK<sup>[46]</sup>.

Since one of the proposed mechanisms for diabetic osteopathy is hyperglycaemia-mediated accumulation of AGEs on bone collagen, and AGEs can decrease osteoblastic maturation and survival via binding to their receptor RAGE, Schurman *et al.*<sup>[47]</sup> investigated whether this process could be modulated *in vitro* by metformin. They found that metformin was able to prevent the increase in apoptosis, caspase 3 activity, inhibition of ALP and alterations in intracellular oxidative stress induced by AGEs in osteoblastic cells, via a metformin-dependent down regulation in the osteoblastic expression of RAGE<sup>[47]</sup>. In another study, Zhen *et al.*<sup>[33]</sup> evaluated whether metformin could prevent the anti-proliferative effect of high-glucose exposure on primary osteoblasts in culture. They found that incubation with metformin decreased the high-glucose-induced intracellular ROS production and apoptosis, and that it additionally induced an osteogenic effect on osteoblasts that was mediated by an increase in Runx2 and IGF-1



**Figure 2 Metformin induces an increase and redistribution of activated AMP-activated protein kinase in multinucleated osteoclasts.** UMR106 osteoblasts and Raw 264.7 macrophages were co-cultured for 7 d, in the absence (A) or presence (B) of 500 mol/L metformin. Cells were fixed, permeabilized and incubated with an anti-phosphorylated AMPK antibody, followed by a FITC-conjugated secondary antibody (green). Nuclei were counterstained with propidium iodide (red). Cells were visualized with a Leica TSC SP5 AOBS confocal microscope. Metformin induced an increase in activated AMPK with a punctillate and predominantly cytoplasmic distribution. AMPK: AMP-activated protein kinase.

gene expression<sup>[33]</sup>. These results have been recently confirmed by other investigators<sup>[48]</sup>.

Three different studies have investigated the *in vitro* effects of metformin on stromal cells isolated from bone marrow (MSC). In the first report, Gao *et al.*<sup>[49]</sup> found that metformin increased Runx2 and decreased PPAR $\gamma$  expression, and consequently stimulated ALP and mineralization while inhibiting the intracellular accumulation of lipid droplets. These results suggest that metformin could influence the reciprocal relationship between osteoblastic and adipogenic differentiation of MSC, tipping the balance towards osteogenesis. Molinuevo *et al.*<sup>[37]</sup> further demonstrated that metformin could induce an *in vitro* dose-dependent increase in MSC osteogenic potential (ALP, type 1 collagen secretion and mineralization). They also found that metformin dose-dependently prevents rosiglitazone-induced intracellular lipid accumulation by MSC<sup>[37]</sup>. In another study, Sedlinsky *et al.*<sup>[50]</sup> demonstrated that the *in vitro* osteogenic effect of metformin on MSC is AMPK-dependent, and that it can be completely blocked by the AMPK inhibitor compound C.

Metformin has also been found to modulate *in vitro* osteoclast recruitment, differentiation and bone-resorbing activity in some<sup>[35,46,51]</sup> but not all<sup>[52]</sup> published reports. OPG and RANKL are predominantly secreted by osteoblasts and play critical roles in osteoclast physiology. As stated above, *in vitro* experiments have shown that metformin increases OPG and reduces RANKL mRNA and protein expression by osteoblasts, which is potentially anti-osteoclastogenic. Additionally, when a macrophage cell line was incubated with the supernatant of osteoblasts treated with metformin, this reduced the formation of tartrate resistant acid phosphatase (TRAP)-positive multi-nucleated osteoclasts<sup>[46]</sup>. In another interesting *in vitro* study, AMPK was found to be expressed by bone marrow pre-osteoclasts and as such is a regulatory target for osteoclast differentiation and resorptive activity. Pharmacological inhibition of pre-osteoclastic AMPK with compound

C increased the RANKL-induced formation of TRAP-positive multinucleated cells and their resorptive activity on dentine discs, *via* downstream activation of p38, JNK, NF- $\kappa$ B, Akt, CREB, c-Fos, and NFATc1. On the contrary, metformin dose-dependently suppressed formation of TRAP-positive multinucleated cells and dentine resorption<sup>[35]</sup>. In unpublished results using indirect immunofluorescence, we have found a metformin-induced increase and sub-cellular redistribution in phosphorylated (activated) AMPK of multinucleated osteoclasts obtained from osteoblast-macrophage co-cultures (Figure 2), which could be mediating the effects of metformin in this cell type.

All in all, these *in vitro* results point to a global bone-anabolic effect of metformin: Tipping the phenotypic balance of bone MSC towards osteoblastogenesis, increasing the bone-forming capacity of osteoblasts, and decreasing the recruitment and bone-resorbing activity of osteoclasts (Figure 3). These findings are further supported by *in vivo* and *ex vivo* (pre-clinical) as well as clinical evidence, pointing to an osteogenic action of metformin in the context of DM.

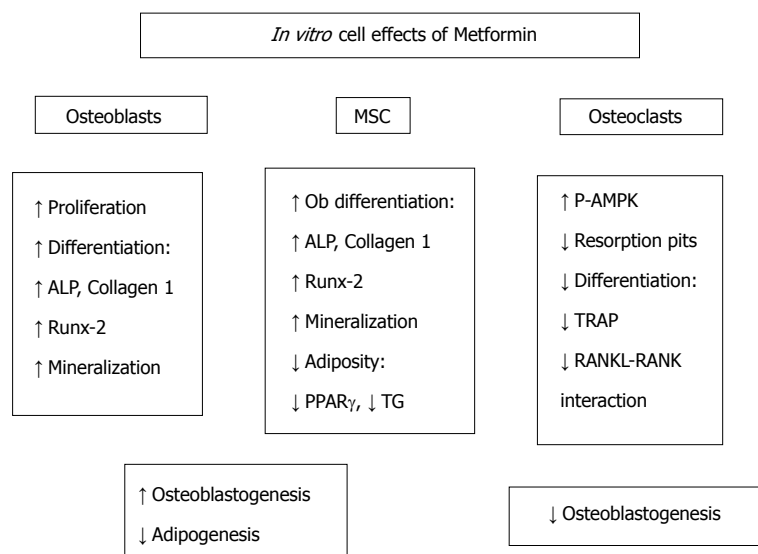
#### ***In vivo and ex vivo effects of metformin on bone metabolism: Animal models***

Most studies using animal models (but not all) have shown beneficial actions of metformin on bone metabolism and on bone lesion repair (Figure 4).

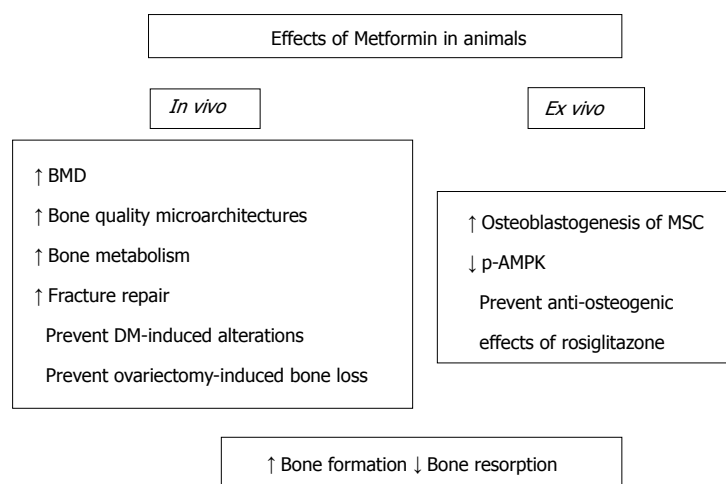
Molinuevo *et al.*<sup>[37]</sup> demonstrated an *ex vivo* osteogenic effect of metformin: *i.e.*, that bone MSC obtained from rats after a 2-wk treatment with oral metformin, exhibit increased osteogenic potential (Runx2 expression, ALP activity, type 1 collagen production, osteocalcin synthesis, and mineral nodule deposition) vs MSC obtained from non-treated animals. In addition, these metformin-induced effects were found to be secondary to AMPK activation. In this study, metformin treatment also stimulated the repair of a minimal parietal lesion *in vivo*, both in diabetic and non-diabetic rats.

As stated above, rosiglitazone is a TZD that induces





**Figure 3 Effects of metformin on bone-derived cells.** The results of several *in vitro* studies show that metformin modulates the phenotypic balance of bone marrow stromal cells (MSC) away from adipogenesis and towards osteoblastogenesis. In addition, metformin increases *in vitro* the bone-forming capacity of osteoblasts, while decreasing the recruitment and bone-resorbing activity of osteoclasts. ALP: Alkaline phosphatase; TG: Triglycerides; TRAP: Tartrate-resistant acid phosphatase; RANK: Receptor activator for nuclear factor  $\kappa$ B; RANKL: RANK ligand.



**Figure 4 Actions of metformin on bone metabolism - animal studies.** Orally administered metformin promotes the osteogenic potential of bone MSC, increases the quality of bone tissue (improving its micro-architecture and mineral density) and facilitates the repair of bone lesions. In addition, metformin may prevent experimental diabetic osteopathy as well as ovariectomy-induced bone loss. MSC: Marrow stromal cells; BMD: Bone mineral density; p-AMPK: Phosphorylated AMP-activated kinase.

deleterious effects on osteoblast differentiation<sup>[53]</sup> and on osteocyte survival<sup>[54]</sup>, diverting MSC differentiation toward the adipocyte lineage. In view of the opposite effect that has been demonstrated for metformin on these cell types, Sedlinsky *et al.*<sup>[50]</sup> investigated the effect of a 2-wk metformin/rosiglitazone combined oral treatment of rats on long-bone metaphyseal microarchitecture, minimal parietal lesion repair and MSC osteogenic potential. Compared to untreated controls, rosiglitazone monotherapy decreased femoral metaphysis trabecular area, osteoblastic and osteocytic density, and TRAP activity associated with epiphyseal growth plates. In addition, it greatly diminished bone repair. It also decreased the *ex vivo* osteogenic potential of MSC, inducing an increase in PPAR<sub>γ</sub> and a

decrease in Runx2 expression, as well as a decrease in phosphorylated (active) AMPK. Metformin/rosiglitazone co-treatment prevented all the *in vivo* (bone repair and diaphyseal microarchitecture) and *ex vivo* anti-osteogenic effects of rosiglitazone monotherapy, with a reversion back to control levels of PPAR<sub>γ</sub>, Runx2 and AMPK phosphorylation in MSC<sup>[50]</sup>.

In another study, the skeletal (femoral) effects of rosiglitazone were compared to those of metformin in insulin-resistant female C57BL6J ob/ob mice. The metformin-treated group showed higher BMD, higher trabecular bone volume/total bone volume, higher osteoid width and mineral apposition, lower trabecular spacing and lower bone marrow adiposity, when compared with the rosiglitazone-treated group<sup>[55]</sup>.

Gao *et al.*<sup>[56]</sup> studied the *in vivo* effect of oral metformin on bone mass in ovariectomized rats. They found that metformin dose-dependently reverted ovariectomy-induced bone loss, showing an improvement in BMD measured by DEXA, and in bone microarchitecture measured both by micro CT and by bone histology. By real-time PCR of MSC, they found a metformin-dependent increase in Runx2 and Lrp5 (co-receptor for Wnt) expression, both of which are involved in osteoblastic proliferation and differentiation<sup>[56]</sup>.

In another report, Jeyabalan *et al.*<sup>[57]</sup> studied either ovariectomized C57BL/6 mice or young Wistar rats to evaluate the effect of oral metformin on bone metabolism and fracture repair, respectively. In both models, metformin did not modify bone microarchitecture or cellular activity *in vivo* as evaluated by micro-CT and bone histomorphometry. In addition, metformin had no significant effect on the repair of a midshaft femoral fracture in Wistar rats<sup>[57]</sup>.

Mai *et al.*<sup>[46]</sup> further investigated the effects on bone of an oral metformin treatment in ovariectomized (OVX) adult rats. They found that metformin treatment of OVX animals significantly increased total body BMD, enhanced bone mineral content and decreased trabecular separation; supporting the concept that metformin can prevent OVX-induced bone loss. The authors also found that metformin reverted the OVX-associated increase in TRAP-positive osteoclasts of proximal tibiae resorption pits. Metformin treatment also increased serum OPG, and decreased RANKL expression by MSC, in OVX rats. Further *in vitro* experiments showed that these effects were regulated by AMPK and by its upstream activator CaMKK.

In a rat model of partially insulin-deficient nicotinamide/streptozotocin-induced DM, tibia histomorphometry showed a diabetes-induced decrease in trabecular bone volume, osteocyte density, growth plate height and osteoclast (TRAP positive) activity in the primary spongiosa, as well as an increase in bone marrow adiposity. MSC from diabetic animals showed a decrease in their osteoblastic potential, an increase in adipocytic commitment, a reduction in their Runx2/PPAR $\gamma$  ratio and an increased expression of the AGEs receptor RAGE. A 2-wk oral treatment with metformin prevented all these Diabetes-induced alterations in bone micro-architecture and MSC osteogenic potential, and also induced a down-regulation of RAGE expression by MSC<sup>[58]</sup>.

### Clinical evidences of metformin effects on bone

There are few published clinical studies reporting the skeletal effects of metformin. In addition, randomized placebo-controlled studies in humans that evaluate the effects of metformin on bone metabolism as a primary end point are so far unavailable. The results of published clinical reports are summarized in Table 2.

Several epidemiological studies have reported the effects of diabetes and antidiabetic agents on bone fracture risk. In 2005, Vestergaard *et al.*<sup>[59]</sup> published

a Danish population-based study evaluating risk of fractures and its relationship with T1DM and T2DM and anti-diabetic agents. They found that both T1DM and T2DM patients had a significant increase in bone fracture risk, and that the use of metformin was associated with a significantly decreased risk for fracture at any site.

Melton *et al.*<sup>[6]</sup> conducted another population-based study in Rochester, United States, to evaluate fracture risk factors in T2DM patients. They found that patients had an increased risk of hip fracture after 10 years of DM, and that use of biguanides such as metformin was protective even after adjusting for other risk factors (HR, 0.7; 95%CI: 0.6-0.96).

Monami *et al.*<sup>[60]</sup> conducted a case-control study, nested within a retrospective cohort, comparing 83 case subjects with a history of bone fractures and 249 control subjects, in all cases exposed to insulin, insulin secretagogues or metformin treatment for the past 10 years, in order to assess the risk for bone fractures associated with exposure to insulin or different oral hypoglycaemic agents. This study was unable to demonstrate a reduction in bone fractures associated with metformin treatment, but showed an increased rate of fractures in patients on insulin treatment, probably related to worse diabetes control or to hypoglycaemic episodes. Nevertheless, the authors acknowledged that the lack of a statistically significant fracture reduction associated with metformin treatment was probably related to an insufficient sample size.

The ADOPT study (A Diabetes Outcome Progression Trial), that compared the glycaemic effects of rosiglitazone, metformin and glyburide, showed that among the adverse effects of rosiglitazone was an increased risk of fracture in women. At the same time they showed that metformin had a lower risk of fracture, both in women and men, for every skeletal site assessed<sup>[12]</sup>. In an add-on report to the ADOPT study, C-telopeptide levels (CTX, a bone resorption marker) were found to be reduced by metformin treatment and increased in rosiglitazone-treated patients, suggesting that changes in bone resorption may be partly responsible for the differences in fracture risk observed for both treatments<sup>[61]</sup>.

A randomized, parallel group, double-blind, multicentre study comparing the efficacy and safety of rosiglitazone/metformin co-treatment (RSG/MET) vs metformin monotherapy (MET) was conducted in order to assess glycaemic control and BMD after 80 wk of treatment in drug-naïve T2DM patients. Although the RSG/MET combination was superior to MET in achieving significant reductions in glycated haemoglobin and fasting plasma glucose, RSG/MET was associated with a significantly lower BMD in comparison with MET at week 80 in the hip and lumbar spine<sup>[62]</sup>.

In another study, Hegazy<sup>[63]</sup> evaluated the possible anti-osteoporotic effect of metformin vs sitagliptin in 40 post-menopausal diabetic women. They were randomly divided into two groups, one receiving 500 mg

**Table 2 Clinical evidences of metformin effects on bone**

Study design	Study population	n	Outcome	Ref.
Case control study	All subjects with bone fracture in Denmark (year 2000), <i>vs</i> 3-fold controls	124655 fracture patients 373962 control	Fracture risk = 0.81 (95%CI: 0.70-0.93) <sup>1</sup> (for metformin)	[59]
Cohort Study	Rochester residents first meeting Diabetes glycaemic criteria (1970-1994)	1964 diabetic patients	Fracture risk = 0.7 (95%CI: 0.6-0.96) <sup>2</sup> (for metformin)	[6]
Case control study	A study nested within a cohort of 1945 diabetic Tuscany outpatients (1998-2004)	83 fracture patients 249 control	Fracture risk = 0.60 (95%CI: 0.34-1.08) <sup>3</sup> (for metformin)	[60]
Double-blind, randomized, controlled clinical trial	Recently diagnosed, drug-naïve patients with type 2 diabetes, treated for a median of 4 yr with rosiglitazone, metformin, or glyburide	Rosiglitazone: n = 1456; Metformin: n = 1454; Glyburide: n = 1441	N <sup>o</sup> Fractures (%): Rosiglitazone 60 (9.30) Metformin 30 (5.08) <sup>4</sup> Glyburide 21 (3.47) <sup>4</sup>	[12]
Double-blind, randomized, controlled clinical trial	Recently diagnosed, drug-naïve patients with type 2 diabetes, treated for a median of 4 yr with RSG, MET, or GLY	Paired baseline and 12-mo stored serum samples from 1605 patients	In women, CTX increased by 6.1% with RSG, decreased by 1.3% with MET (P = 0.03) In men, CTX was unchanged on RSG (-1.0%) and fell with MET -12.7% (P = 0.001)	[61]
Randomized, parallel group, double-blind, multicentre study	Drug naïve, male and female patients who had an established clinical diagnosis of type 2 diabetes mellitus	688 patients equally randomized to RSG/MET or MET	BMD at week 80: Lumbar = (-2.2) (95%CI: -3.5, -0.9) Total hip = (-1.5) (95%CI: -2.3, -0.7) <sup>5</sup>	[62]
Prospective randomized study with active comparator study	Forty postmenopausal diabetic women recruited from Tanta University Hospitals	20 patients on metformin and 20 on sitagliptin, for 12 wk	BMD was unchanged in both groups at week 12 Bone turnover markers remained unchanged from baseline in MET	[63]
Prospective randomized double-blind, double-dummy with active comparator	Men with uncomplicated type 2 diabetes mellitus, aged 45-65 yr	71 men were randomized to PIO once daily or MET twice daily	Sclerostin levels at week 24 increased by 11% in PIO-treated patients and decreased by 1.8% in MET-treated patients (P = 0.018)	[64]

<sup>1</sup>Relative risk of any fracture interpreted as OR with 95%CI for several variables in the population of Denmark (National Health Registry, year 2000);

<sup>2</sup>Multivariate HR for the development of any new fracture among 1964 Rochester, MN, United States residents after recognition of diabetes mellitus in 1970-1994; <sup>3</sup>Exposure for at least 36 mo to hypoglycemic treatments in case subjects and matched control subjects, interpreted as OR with 95%CI; <sup>4</sup>P < 0.01 for comparison of fracture risk in women with rosiglitazone (unadjusted, contingency  $\chi^2$  test); <sup>5</sup>Percentage of change in BMD at week 80, comparing RSG/MET *vs* MET. RSG: Rosiglitazone; MET: Metformin; GLY: Glyburide; PIO: Pioglitazone; BMD: Bone mineral density; OR: Odds ratio; HR: Hazard ratios.

metformin twice a day, and the other 100 mg sitagliptin once a day, for 12 wk. In the metformin-treated group, serum ALP and urinary D-piridinoline (DPD) were not significantly different from baseline; conversely in the sitagliptin group, serum ALP and urinary DPD decreased significantly after 12 wk, although BMD was unchanged in both groups.

The effects of pioglitazone and metformin on circulating sclerostin (an osteocyte-derived osteoblast proliferation inhibitor), and biochemical markers of bone turnover were studied in 71 men with T2DM. This group as a whole showed higher serum sclerostin levels than healthy controls. Sclerostin levels were further increased in the sub-set of patients that were treated with pioglitazone, who also showed an increase in serum CTX. On the contrary, metformin-treated patients *vs* healthy controls showed significantly lower sclerostin levels and unchanged CTX levels<sup>[64]</sup>. Although sclerostin is a well-established inhibitor of bone formation, recent evidence indicates that it can also promote osteoclastogenesis by stimulating RANKL produced by osteocytes<sup>[65]</sup>, suggesting that pioglitazone could increase bone resorption while decreasing bone formation, and the opposite would occur with metformin.

Finally, metformin was tested for bone-defect healing purposes in a clinical study, adding this biguanide to

platelet-rich fibrin in order to treat intrabony defects in patients with chronic periodontitis. The study was designed to evaluate the efficacy of platelet-rich fibrin, 1% metformin gel, or platelet-rich fibrin plus 1% metformin gel, in all cases with open flap debridement, for treatment of intrabony defects in 120 patients with chronic periodontitis. The group treated with platelet-rich fibrin plus 1% metformin gel showed the greatest improvements in clinical parameters, with an increase in percentage radiographic defect depth reduction when compared to metformin alone, platelet-rich fibrin alone or open flap debridement alone<sup>[66]</sup>.

## CONCLUSION AND PERSPECTIVES

Patients with long-term T1 DM and T2 DM can develop skeletal complications or "diabetic osteopathy". These include osteopenia, osteoporosis and an increased incidence of low-stress fractures. In this context, it is important to evaluate whether current anti-diabetic treatments can secondarily affect bone metabolism. Over the past 10 years, many investigators have studied the effects of metformin on bone, providing a considerable body of pre-clinical (*in vitro*, *ex vivo* and *in vivo*) as well as clinical evidence for an anabolic action of metformin on bone. However three reports (one *in vitro*, one *in vivo*, one clinical) have been unable to link

metformin treatment with bone anabolic processes, underscoring the differences that exist between experimental models in pre-clinical studies, and the low statistical potency inherent in clinical reports that include a relatively small number of patients. In this sense, two caveats should be kept in mind when considering metformin treatment for a patient with T2DM at risk for diabetic osteopathy. In the first place, metformin should probably not be considered an anti-osteoporotic drug; it is an insulin sensitizer with proven macrovascular benefits that can secondarily improve bone metabolism in the context of DM. Secondly, we are still awaiting the results of randomized placebo-controlled studies in humans that evaluate the effects of metformin on bone metabolism as a primary endpoint.

## REFERENCES

- 1 McCarthy AD, Molinuevo MS, Cortizo AM. AGEs and Bone Ageing in Diabetes Mellitus. *J Diabetes Metab* 2013; **4**: 276 [DOI: 10.4172/2155-6156.1000276]
- 2 Bouillon R. Diabetic bone disease. *Calcif Tissue Int* 1991; **49**: 155-160 [PMID: 1933578]
- 3 Hofbauer LC, Brueck CC, Singh SK, Dobnig H. Osteoporosis in patients with diabetes mellitus. *J Bone Miner Res* 2007; **22**: 1317-1328 [PMID: 17501667]
- 4 Forsén L, Meyer HE, Midthjell K, Edna TH. Diabetes mellitus and the incidence of hip fracture: results from the Nord-Trøndelag Health Survey. *Diabetologia* 1999; **42**: 920-925 [PMID: 10491750]
- 5 Nicodemus KK, Folsom AR. Type 1 and type 2 diabetes and incident hip fractures in postmenopausal women. *Diabetes Care* 2001; **24**: 1192-1197 [PMID: 11423501]
- 6 Melton LJ, Leibson CL, Achenbach SJ, Therneau TM, Khosla S. Fracture risk in type 2 diabetes: update of a population-based study. *J Bone Miner Res* 2008; **23**: 1334-1342 [PMID: 18348689 DOI: 10.1359/jbmr.080323]
- 7 Schwartz AV, Sellmeyer DE, Ensrud KE, Cauley JA, Tabor HK, Schreiner PJ, Jamal SA, Black DM, Cummings SR. Older women with diabetes have an increased risk of fracture: a prospective study. *J Clin Endocrinol Metab* 2001; **86**: 32-38 [PMID: 11231974]
- 8 Silva MJ, Brodt MD, Lynch MA, McKenzie JA, Tanouye KM, Nyman JS, Wang X. Type 1 diabetes in young rats leads to progressive trabecular bone loss, cessation of cortical bone growth, and diminished whole bone strength and fatigue life. *J Bone Miner Res* 2009; **24**: 1618-1627 [PMID: 19338453 DOI: 10.1359/jbmr.090316]
- 9 Saito M, Marumo K. Collagen cross-links as a determinant of bone quality: a possible explanation for bone fragility in aging, osteoporosis, and diabetes mellitus. *Osteoporos Int* 2010; **21**: 195-214 [PMID: 19760059 DOI: 10.1007/s00198-009-1066-z]
- 10 Gilbert MP, Pratley RE. The impact of diabetes and diabetes medications on bone health. *Endocr Rev* 2015; **36**: 194-213 [PMID: 25738213 DOI: 10.1210/er.2012-1042]
- 11 Ali AA, Weinstein RS, Stewart SA, Parfitt AM, Manolagas SC, Jilka RL. Rosiglitazone causes bone loss in mice by suppressing osteoblast differentiation and bone formation. *Endocrinology* 2005; **146**: 1226-1235 [PMID: 15591153]
- 12 Kahn SE, Haffner SM, Heise MA, Herman WH, Holman RR, Jones NP, Kravitz BG, Lachin JM, O'Neill MC, Zinman B, Viberti G. Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. *N Engl J Med* 2006; **355**: 2427-2443 [PMID: 17145742]
- 13 Monami M, Dicembrini I, Antenore A, Mannucci E. Dipeptidyl peptidase-4 inhibitors and bone fractures: a meta-analysis of randomized clinical trials. *Diabetes Care* 2011; **34**: 2474-2476 [PMID: 22025784 DOI: 10.2337/dc11-1099]
- 14 Sbaraglini ML, Molinuevo MS, Sedlinsky C, Schurman L, McCarthy AD. Saxagliptin affects long-bone microarchitecture and decreases the osteogenic potential of bone marrow stromal cells. *Eur J Pharmacol* 2014; **727**: 8-14 [PMID: 24485890 DOI: 10.1016/j.ejphar.2014.01.028]
- 15 Taylor SI, Blau JE, Rother KI. Possible adverse effects of SGLT2 inhibitors on bone. *Lancet Diabetes Endocrinol* 2015; **3**: 8-10 [PMID: 25523498 DOI: 10.1016/S2213-8587(14)70227-X]
- 16 Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. *Lancet* 1998; **352**: 854-865 [PMID: 9742977]
- 17 Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, Wu M, Ventre J, Doebber T, Fujii N, Musi N, Hirshman MF, Goodyear LJ, Moller DE. Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest* 2001; **108**: 1167-1174 [PMID: 11602624]
- 18 Musi N, Hirshman MF, Nygren J, Svanfeldt M, Bavenholm P, Rooyackers O, Zhou G, Williamson JM, Ljunqvist O, Efendic S, Moller DE, Thorell A, Goodyear LJ. Metformin increases AMP-activated protein kinase activity in skeletal muscle of subjects with type 2 diabetes. *Diabetes* 2002; **51**: 2074-2081 [PMID: 12086935]
- 19 Shah M, Kola B, Batavljic A, Arnett TR, Viollet B, Saxon L, Korbonsits M, Chenu C. AMP-activated protein kinase (AMPK) activation regulates in vitro bone formation and bone mass. *Bone* 2010; **47**: 309-319 [PMID: 20399918 DOI: 10.1016/j.bone.2010.04.596]
- 20 Sanz P. AMP-activated protein kinase: structure and regulation. *Curr Protein Pept Sci* 2008; **9**: 478-492 [PMID: 18855699]
- 21 Mounier R, Thérêt M, Lantier L, Foretz M, Viollet B. Expanding roles for AMPK in skeletal muscle plasticity. *Trends Endocrinol Metab* 2015; **26**: 275-286 [PMID: 25818360 DOI: 10.1016/j.tem.2015.02.009]
- 22 Strack T. Metformin: a review. *Drugs Today (Barc)* 2008; **44**: 303-314 [PMID: 18536788]
- 23 Carling D, Viollet B. Beyond energy homeostasis: the expanding role of AMP-activated protein kinase in regulating metabolism. *Cell Metab* 2015; **21**: 799-804 [PMID: 26039446 DOI: 10.1016/j.cmet.2015.05.005]
- 24 Xie M, Roy R. AMP-Activated Kinase Regulates Lipid Droplet Localization and Stability of Adipose Triglyceride Lipase in *C. elegans* Dauer Larvae. *PLoS One* 2015; **10**: e0130480 [PMID: 26098762 DOI: 10.1371/journal.pone.0130480]
- 25 Bijland S, Mancini SJ, Salt IP. Role of AMP-activated protein kinase in adipose tissue metabolism and inflammation. *Clin Sci (Lond)* 2013; **124**: 491-507 [PMID: 23298225 DOI: 10.1042/CS20120536]
- 26 Ruderman NB, Carling D, Prentki M, Cacicedo JM. AMPK, insulin resistance, and the metabolic syndrome. *J Clin Invest* 2013; **123**: 2764-2772 [PMID: 23863634 DOI: 10.1172/JCI67227]
- 27 Viollet B, Andreelli F, Jørgensen SB, Perrin C, Flamez D, Mu J, Wojtaszewski JF, Schuit FC, Birnbaum M, Richter E, Burcelin R, Vaulont S. Physiological role of AMP-activated protein kinase (AMPK): insights from knockout mouse models. *Biochem Soc Trans* 2003; **31**: 216-219 [PMID: 12546688]
- 28 Röckl KS, Hirshman MF, Brandauer J, Fujii N, Witters LA, Goodyear LJ. Skeletal muscle adaptation to exercise training: AMP-activated protein kinase mediates muscle fiber type shift. *Diabetes* 2007; **56**: 2062-2069 [PMID: 17513699]
- 29 Saclier M, Cuvellier S, Magnan M, Mounier R, Chazaud B. Monocyte/macrophage interactions with myogenic precursor cells during skeletal muscle regeneration. *FEBS J* 2013; **280**: 4118-4130 [PMID: 23384231 DOI: 10.1111/febs.12166]
- 30 Jeyabalan J, Shah M, Viollet B, Chenu C. AMP-activated protein kinase pathway and bone metabolism. *J Endocrinol* 2012; **212**: 277-290 [PMID: 21903861 DOI: 10.1530/JOE-11-0306]
- 31 Kang H, Viollet B, Wu D. Genetic deletion of catalytic subunits of AMP-activated protein kinase increases osteoclasts and reduces bone mass in young adult mice. *J Biol Chem* 2013; **288**: 12187-12196 [PMID: 23486478 DOI: 10.1074/jbc.M112.430389]
- 32 Kanazawa I, Yamaguchi T, Yano S, Yamauchi M, Yamamoto M, Sugimoto T. Adiponectin and AMP kinase activator stimulate



- proliferation, differentiation, and mineralization of osteoblastic MC3T3-E1 cells. *BMC Cell Biol* 2007; **8**: 51 [PMID: 18047638]
- 33 **Zhen D**, Chen Y, Tang X. Metformin reverses the deleterious effects of high glucose on osteoblast function. *J Diabetes Complications* 2010; **24**: 334-344 [PMID: 19628413 DOI: 10.1016/j.jdiacomp.2009.05.002]
- 34 **Kasai T**, Badow K, Suzuki H, Chiba N, Kakimoto K, Ohnishi T, Kawamoto S, Nagaoka E, Matsuguchi T. Osteoblast differentiation is functionally associated with decreased AMP kinase activity. *J Cell Physiol* 2009; **221**: 740-749 [PMID: 19725053 DOI: 10.1002/jcp.21917]
- 35 **Lee YS**, Kim YS, Lee SY, Kim GH, Kim BJ, Lee SH, Lee KU, Kim GS, Kim SW, Koh JM. AMP kinase acts as a negative regulator of RANKL in the differentiation of osteoclasts. *Bone* 2010; **47**: 926-937 [PMID: 20696287 DOI: 10.1016/j.bone.2010.08.001]
- 36 **Zhao J**, Yue W, Zhu MJ, Sreejayan N, Du M. AMP-activated protein kinase (AMPK) cross-talks with canonical Wnt signaling via phosphorylation of beta-catenin at Ser 552. *Biochem Biophys Res Commun* 2010; **395**: 146-151 [PMID: 20361929 DOI: 10.1016/j.bbrc.2010.03.161]
- 37 **Molinuevo MS**, Schurman L, McCarthy AD, Cortizo AM, Tolosa MJ, Gangoiti MV, Arnol V, Sedlinsky C. Effect of metformin on bone marrow progenitor cell differentiation: in vivo and in vitro studies. *J Bone Miner Res* 2010; **25**: 211-221 [PMID: 19594306 DOI: 10.1359/jbmr.090732]
- 38 **Foretz M**, Hébrard S, Leclerc J, Zarrinpashneh E, Soty M, Mithieux G, Sakamoto K, Andreelli F, Viollet B. Metformin inhibits hepatic gluconeogenesis in mice independently of the LKB1/AMPK pathway via a decrease in hepatic energy state. *J Clin Invest* 2010; **120**: 2355-2369 [PMID: 20577053 DOI: 10.1172/JCI40671]
- 39 **Viollet B**, Guigas B, Sanz Garcia N, Leclerc J, Foretz M, Andreelli F. Cellular and molecular mechanisms of metformin: an overview. *Clin Sci (Lond)* 2012; **122**: 253-270 [PMID: 22117616 DOI: 10.1042/CS20110386]
- 40 **Ouslimani N**, Peynet J, Bonnefont-Rousselot D, Théron P, Legrand A, Beaudeau JL. Metformin decreases intracellular production of reactive oxygen species in aortic endothelial cells. *Metabolism* 2005; **54**: 829-834 [PMID: 15931622]
- 41 **Cortizo AM**, Sedlinsky C, McCarthy AD, Blanco A, Schurman L. Osteogenic actions of the anti-diabetic drug metformin on osteoblasts in culture. *Eur J Pharmacol* 2006; **536**: 38-46 [PMID: 16564524]
- 42 **Kanazawa I**, Yamaguchi T, Yano S, Yamauchi M, Sugimoto T. Metformin enhances the differentiation and mineralization of osteoblastic MC3T3-E1 cells via AMP kinase activation as well as eNOS and BMP-2 expression. *Biochem Biophys Res Commun* 2008; **375**: 414-419 [PMID: 18721796 DOI: 10.1016/j.bbrc.2008.08.034]
- 43 **Jang WG**, Kim EJ, Lee KN, Son HJ, Koh JT. AMP-activated protein kinase (AMPK) positively regulates osteoblast differentiation via induction of Dlx5-dependent Runx2 expression in MC3T3E1 cells. *Biochem Biophys Res Commun* 2011; **404**: 1004-1009 [PMID: 21187071 DOI: 10.1016/j.bbrc.2010.12.099]
- 44 **Salai M**, Somjen D, Gigi R, Yakobson O, Katzburg S, Dolkart O. Effects of commonly used medications on bone tissue mineralisation in SaOS-2 human bone cell line: an in vitro study. *Bone Joint J* 2013; **95-B**: 1575-1580 [PMID: 24151282 DOI: 10.1302/0301-620X.95B11.31158]
- 45 **Jang WG**, Kim EJ, Bae IH, Lee KN, Kim YD, Kim DK, Kim SH, Lee CH, Franceschi RT, Choi HS, Koh JT. Metformin induces osteoblast differentiation via orphan nuclear receptor SHP-mediated transactivation of Runx2. *Bone* 2011; **48**: 885-893 [PMID: 21147283 DOI: 10.1016/j.bone.2010.12.003]
- 46 **Mai QG**, Zhang ZM, Xu S, Lu M, Zhou RP, Zhao L, Jia CH, Wen ZH, Jin DD, Bai XC. Metformin stimulates osteoprotegerin and reduces RANKL expression in osteoblasts and ovariectomized rats. *J Cell Biochem* 2011; **112**: 2902-2909 [PMID: 21618594 DOI: 10.1002/jcb.23206]
- 47 **Schurman L**, McCarthy AD, Sedlinsky C, Gangoiti MV, Arnol V, Bruzzzone L, Cortizo AM. Metformin reverts deleterious effects of advanced glycation end-products (AGEs) on osteoblastic cells. *Exp Clin Endocrinol Diabetes* 2008; **116**: 333-340 [PMID: 18273753 DOI: 10.1055/s-2007-992786]
- 48 **Shao X**, Cao X, Song G, Zhao Y, Shi B. Metformin rescues the MG63 osteoblasts against the effect of high glucose on proliferation. *J Diabetes Res* 2014; **2014**: 453940 [PMID: 24812633 DOI: 10.1155/2014/453940]
- 49 **Gao Y**, Xue J, Li X, Jia Y, Hu J. Metformin regulates osteoblast and adipocyte differentiation of rat mesenchymal stem cells. *J Pharm Pharmacol* 2008; **60**: 1695-1700 [PMID: 19000376 DOI: 10.1211/jpp.60/12.0017]
- 50 **Sedlinsky C**, Molinuevo MS, Cortizo AM, Tolosa MJ, Felice JJ, Sbaraglini ML, Schurman L, McCarthy AD. Metformin prevents anti-osteogenic in vivo and ex vivo effects of rosiglitazone in rats. *Eur J Pharmacol* 2011; **668**: 477-485 [PMID: 21839072 DOI: 10.1016/j.ejphar.2011.07.033]
- 51 **Son HJ**, Lee J, Lee SY, Kim EK, Park MJ, Kim KW, Park SH, Cho ML. Metformin attenuates experimental autoimmune arthritis through reciprocal regulation of Th17/Treg balance and osteoclastogenesis. *Mediators Inflamm* 2014; **2014**: 973986 [PMID: 25214721 DOI: 10.1155/2014/973986]
- 52 **Patel JJ**, Butters OR, Arnett TR. PPAR agonists stimulate adipogenesis at the expense of osteoblast differentiation while inhibiting osteoclast formation and activity. *Cell Biochem Funct* 2014; **32**: 368-377 [PMID: 24615887 DOI: 10.1002/cbf.3025]
- 53 **Lecka-Czernik B**, Moerman EJ, Grant DF, Lehmann JM, Manolagas SC, Jilka RL. Divergent effects of selective peroxisome proliferator-activated receptor-gamma 2 ligands on adipocyte versus osteoblast differentiation. *Endocrinology* 2002; **143**: 2376-2384 [PMID: 12021203]
- 54 **Rzonca SO**, Suva LJ, Gaddy D, Montague DC, Lecka-Czernik B. Bone is a target for the antidiabetic compound rosiglitazone. *Endocrinology* 2004; **145**: 401-406 [PMID: 14500573]
- 55 **Wang C**, Li H, Chen SG, He JW, Sheng CJ, Cheng XY, Qu S, Wang KS, Lu ML, Yu YC. The skeletal effects of thiazolidinedione and metformin on insulin-resistant mice. *J Bone Miner Metab* 2012; **30**: 630-637 [PMID: 22886403 DOI: 10.1007/s00774-012-0374-0]
- 56 **Gao Y**, Li Y, Xue J, Jia Y, Hu J. Effect of the anti-diabetic drug metformin on bone mass in ovariectomized rats. *Eur J Pharmacol* 2010; **635**: 231-236 [PMID: 20307532 DOI: 10.1016/j.ejphar.2010.02.051]
- 57 **Jeyabalan J**, Viollet B, Smitham P, Ellis SA, Zaman G, Bardin C, Goodship A, Roux JP, Pierre M, Chenu C. The anti-diabetic drug metformin does not affect bone mass in vivo or fracture healing. *Osteoporos Int* 2013; **24**: 2659-2670 [PMID: 23644877 DOI: 10.1007/s00198-013-2371-0]
- 58 **Tolosa MJ**, Chuguransky SR, Sedlinsky C, Schurman L, McCarthy AD, Molinuevo MS, Cortizo AM. Insulin-deficient diabetes-induced bone microarchitecture alterations are associated with a decrease in the osteogenic potential of bone marrow progenitor cells: preventive effects of metformin. *Diabetes Res Clin Pract* 2013; **101**: 177-186 [PMID: 23806481 DOI: 10.1016/j.diabres.2013.05.016]
- 59 **Vestergaard P**, Rejnmark L, Mosekilde L. Relative fracture risk in patients with diabetes mellitus, and the impact of insulin and oral antidiabetic medication on relative fracture risk. *Diabetologia* 2005; **48**: 1292-1299 [PMID: 15909154]
- 60 **Monami M**, Cresci B, Colombini A, Pala L, Balzi D, Gori F, Chiasserini V, Marchionni N, Rotella CM, Mannucci E. Bone fractures and hypoglycemic treatment in type 2 diabetic patients: a case-control study. *Diabetes Care* 2008; **31**: 199-203 [PMID: 18024851]
- 61 **Zinman B**, Haffner SM, Herman WH, Holman RR, Lachin JM, Kravitz BG, Paul G, Jones NP, Aftring RP, Viberti G, Kahn SE. Effect of rosiglitazone, metformin, and glyburide on bone biomarkers in patients with type 2 diabetes. *J Clin Endocrinol Metab* 2010; **95**: 134-142 [PMID: 19875477 DOI: 10.1210/jc.2009-0572]
- 62 **Borges JL**, Bilezikian JP, Jones-Leone AR, Acosta AP, Ambery PD, Nino AJ, Grosse M, Fitzpatrick LA, Cobitz AR. A randomized, parallel group, double-blind, multicentre study comparing the efficacy and safety of Avandamet (rosiglitazone/metformin) and metformin on long-term glycaemic control and bone mineral

- density after 80 weeks of treatment in drug-naïve type 2 diabetes mellitus patients. *Diabetes Obes Metab* 2011; **13**: 1036-1046 [PMID: 21682834 DOI: 10.1111/j.1463-1326.2011.01461.x]
- 63 **Hegazy SK**. Evaluation of the anti-osteoporotic effects of metformin and sitagliptin in postmenopausal diabetic women. *J Bone Miner Metab* 2015; **33**: 207-212 [PMID: 24633493 DOI: 10.1007/s00774-014-0581-y]
- 64 **van Lierop AH**, Hamdy NA, van der Meer RW, Jonker JT, Lamb HJ, Rijzewijk LJ, Diamant M, Romijn JA, Smit JW, Papapoulos SE. Distinct effects of pioglitazone and metformin on circulating sclerostin and biochemical markers of bone turnover in men with type 2 diabetes mellitus. *Eur J Endocrinol* 2012; **166**: 711-716 [PMID: 22267280 DOI: 10.1530/EJE-11-1061]
- 65 **Wijenayaka AR**, Kogawa M, Lim HP, Bonewald LF, Findlay DM, Atkins GJ. Sclerostin stimulates osteocyte support of osteoclast activity by a RANKL-dependent pathway. *PLoS One* 2011; **6**: e25900 [PMID: 21991382 DOI: 10.1371/journal.pone.0025900]
- 66 **Pradeep AR**, Nagpal K, Karvekar S, Patnaik K, Naik SB, Guruprasad CN. Platelet-rich fibrin with 1% metformin for the treatment of intrabony defects in chronic periodontitis: a randomized controlled clinical trial. *J Periodontol* 2015; **86**: 729-737 [PMID: 25762357 DOI: 10.1902/jop.2015.140646]

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## Infantile onset diabetes mellitus in developing countries - India

Poovazhagi Varadarajan

Poovazhagi Varadarajan, Department of Pediatrics, Chengalpattu Medical College and Hospital, Chengalpattu, Tamil Nadu 603001, India

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Correspondence to: Poovazhagi Varadarajan, Professor of Pediatrics, Department of Pediatrics, Chengalpattu Medical College and Hospital, 8/11 Manjulai Street, Kalaimagal Nagar, Chengalpattu, Tamil Nadu 603001, India. [poomuthu@yahoo.com](mailto:poomuthu@yahoo.com)  
Telephone: +91-44-22251515

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### Abstract

Infantile onset diabetes mellitus (IODM) is an uncommon metabolic disorder in children. Infants with onset of diabetes mellitus (DM) at age less than one year are likely to have transient or permanent neonatal DM or rarely type 1 diabetes. Diabetes with onset below 6 mo is a heterogeneous disease caused by single gene

mutations. Literature on IODM is scanty in India. Nearly 83% of IODM cases present with diabetic keto acidosis at the onset. Missed diagnosis was common in infants with diabetes (67%). Potassium channel mutation with sulphonylurea responsiveness is the common type in the non-syndromic IODM and Wolcott Rallison syndrome is the common type in syndromic diabetes. Developmental delay and seizures were the associated co-morbid states. Genetic diagnosis has made a phenomenal change in the management of IODM. Switching from subcutaneous insulin to oral hypoglycemic drugs is a major clinical breakthrough in the management of certain types of monogenic diabetes. Mortality in neonatal diabetes is 32.5% during follow-up from Indian studies. This article is a review of neonatal diabetes and available literature on IODM from India.

**Key words:** Infants; Diabetes mellitus; Monogenic diabetes; Co-morbid state; Mortality

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**Core tip:** The clinical presentation of infantile onset diabetes mellitus (IODM) as syndromic and non-syndromic forms from South India is discussed in this article. Associated co-morbid states, mortality pattern, difficulty in the management and need for genetic evaluation among this group of infants are also discussed. Identification of this form of monogenic diabetes by clinical evaluation and appropriate genetic evaluation to identify the subtypes helps in the management of these infants to improve the overall morbidity and mortality. Reported mortality in IODM is high from South India.

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## INTRODUCTION

Infantile onset diabetes mellitus (IODM) is a rare form of diabetes with onset at less than one year of age. Onset of diabetes in the first 6 mo of life is termed neonatal diabetes<sup>[1]</sup>. The majority of neonatal diabetes cases are monogenic. However, recent reports have suggested evaluation for monogenic diabetes among those with onset in later infancy (onset between 6 mo and 1 year), too<sup>[2]</sup>. The two phenotypes of neonatal diabetes include transient neonatal diabetes mellitus (TNDM) and permanent neonatal diabetes mellitus (PNDM). The reported incidence of neonatal diabetes varies from 1 in 200000 to 1 in 400000<sup>[3]</sup>. Infants with PNDM continue to require insulin for maintaining euglycemia. TNDM usually resolves by 18 mo of age. In a study from Chennai in India, the incidence of infantile onset diabetes was 7.9% among all diabetic children in a pediatric diabetic clinic<sup>[4]</sup>. Another hospital based retrospective study from South India revealed the incidence to be 7%<sup>[5]</sup>. A recent international cohort study used comprehensive genetic testing to identify causal mutations which were found in nearly 80% of samples in neonatal diabetes<sup>[6]</sup>. This indicates that genetic diagnosis results in a phenomenal change in the management of infantile onset diabetes.

### Genetics of TNDM

Among the neonatal diabetes cases, 50%-60% of affected neonates are due to TNDM based on the Western literature<sup>[7,8]</sup>. However, in a study from Chennai in India, TNDM only accounted for 5% of all neonatal DM cases<sup>[4]</sup>. TNDM is commonly due to a developmental defect in the pancreatic beta cell function. The common genetic defect (60%-70%) is due to mutations on chromosome 6q24<sup>[3,9,10]</sup>. The cause seems to be a defect in maternal methylation, most often due to recessive mutations in the *ZPF57* (Zinc Finger Protein) gene<sup>[11]</sup>. There are three types of abnormalities leading to overexpression of the paternal allele at this locus: (1) a paternally inherited duplication; (2) paternal uniparental disomy (UPD); and (3) an epimutation resulting in a complete loss of methylation of the maternal allele on chromosome 6q24<sup>[12,13]</sup>. Mutations in *KCNJ11* (Potassium channel subfamily J member 11) and *ABCC8* (ATP binding cassette transporter subfamily C, member 8) leading to about 20%-25% of TNDM cases<sup>[13-16]</sup>. Rarely mutations in *HNF 1B*, insulin gene and solute carrier 2 family 2 gene (*SLC2A2*) can result in neonatal diabetes. TNDM remits permanently or may relapse later during adulthood. Thus, TNDM may be a permanent beta cell defect with variable expression during growth and development. The clinical presentation includes hyperglycemia, dehydration, and failure to thrive with or without ketoacidosis. The associated features in chromosome 6q24 mutations include macroglossia (35%), umbilical hernia (14%) or more rarely cardiac and brain developmental defects. Infants with TNDM resulting from a  $K_{ATP}$  channel mutation are often heavier

than patients with chromosome 6q24 induced TNDM at birth, are diagnosed with diabetes later, remit later and relapse earlier<sup>[17]</sup>. Chromosome 6q24 induced TNDM should be treated with insulin. Relapse due to UPD chromosome 6 mutation or *ABCC8* mutation in puberty responds well to sulphonylurea therapy<sup>[18,19]</sup>. Relapsing diabetes due to 6q24 related diabetes has been successfully treated with dipeptidyl peptidase 4 inhibitor<sup>[20]</sup>. The majority (> 90%) of TNDM cases due to *ABCC 8/KCNJ11* mutation respond to sulphonylurea therapy<sup>[21]</sup>. Infants born with TNDM harbour a greater risk of developing type 2 diabetes later in the life<sup>[22]</sup>. There were only few studies, and most were case reports about TNDM in the Indian literature<sup>[23-29]</sup>. Rarely encountered is type 1 diabetes in infancy especially in the non-syndromic diabetes with onset in later part of infancy<sup>[30]</sup>.

### Genetics of PNDM

Children with PNDM have their onset in early infancy and continue to be hyperglycemic, which needs lifelong insulin therapy. The genetic defect involves mutation of genes involving the pancreatic B cell development, function, apoptosis and insulin molecule. Nearly 40% of the defects are in the genes regulating the  $K_{ATP}$  (potassium ATP) channel. As early as 1997 mutations leading to PNDM have been described. The first is pancreatic agenesis due to mutations in *IPF*/pancreatic and duodenal homeobox 1 (*PDX1*). Between 2004 and 2007 the mutations of *KCNJ11*, *ABCC8* and *INS* genes were identified for PNDM. *KCNJ11* and *ABCC8* mutations account for nearly 40%-50% of all PNDM cases<sup>[31]</sup>. PNDM can be nonsyndromic or syndromic (associated with other systemic features). The five genes in which mutations in nonsyndromic PNDM occur include *KCNJ11* (approximately 30% of NDM cases), *ABCC8* (approximately 19%), *INS* (approximately 20%), *GCK* (approximately 4%), and *PDX1* (< 1%)<sup>[32]</sup>. The mode of inheritance of PNDM is autosomal dominant for mutations in *KCNJ11*, autosomal dominant or autosomal recessive for mutations in *ABCC8* and *INS*, and autosomal recessive for mutations in *GCK* and *PDX1*.

## $K_{ATP}$ CHANNEL MUTATION

Glucose sensing and insulin release from beta cells are a complex process. Glucose enters through GLUT receptors and generates energy in the form of ATP. This increased ratio of ATP to ADP results in closing of the potassium channels and depolarization. This activates the calcium channels and influx of calcium into cells, leading to release of insulin by exocytosis. The potassium channel subunit Kir 6.2 (potassium inward receptor) and SUR (sulphonylurea receptor 1) are encoded by genes called *KCNJ11* and *ABCC8*. Mutations in these two genes are common in PNDM. The majority of these children respond to sulphonylurea, which acts on the potassium channels,



keeps them open and prevents depolarization<sup>[33]</sup>. These channels are present in non-pancreatic tissues like the brain, heart and skeletal muscles and this explains the associated co-morbid states like developmental delay, muscle weakness and seizures in DEND syndrome [developmental delay, epilepsy, neonatal diabetes mellitus (NDM)]<sup>[34,35]</sup>. A number of patients with K<sub>ATP</sub> channel mutations present with a milder phenotype without epilepsy [termed intermediate DEND (iDEND) syndrome]<sup>[36-38]</sup>. Infants or children with Kir mutation respond well to sulphonylurea therapy, which is preferred than insulin as sulphonylurea therapy is associated with improvement of other systemic features along with glycemic control<sup>[39]</sup>. Among the published data from South India, out of the 9 identified mutations, 7 were due to *KCNJ11* and *ABCC8* mutations and successful switch was done in these children following genetic reports except one child with *ABCC8* mutation induced hyperinsulinemic hypoglycemia<sup>[40]</sup>.

## INSULIN

*Insulin* gene defects lead to defective folding of insulin in the endoplasmic reticulum and this affects the insulin release. They may present as NDM or MODY<sup>[41]</sup>. These infants do not have any extra-pancreatic features. The heterozygous form presents during the first 6 mo of age and 50% have keto acidosis. The more severe form with homozygous mutation presents much earlier and have low birth weight. Management includes insulin, prevention of hyperglycemia through diet and use of insulin sensitizers like metformin. Sulphonylureas are not effective. Insulin gene mutations leading to neonatal diabetes have been described even in Indian infants<sup>[40,42,43]</sup>.

## GLUCOKINASE

Glucokinase (GCK) is the glucose sensor of the cell. It is essential for phosphorylation of the glucose molecule that enter the cells. Mutations lead to defective glycolytic activity, and thereby the cascade leading to insulin release is affected. Homozygous infants present with NDM. They need lifelong insulin therapy. Heterozygotes may present later as MODY 2 (maturity onset diabetes in young). A homozygous *GCK* mutation has been described in an infant from Chennai<sup>[4,43]</sup>. Although *GCK* mutations in neonates do not respond well to sulphonylurea, there are a few case reports suggesting a role for glibenclamide along with insulin in children with homozygous *GCK* mutations<sup>[44,45]</sup>.

## PDX1

The homozygous form presents as PNDM and has both pancreatic exocrine and endocrine dysfunctions. The heterozygous form present as MODY 4<sup>[46]</sup>.

Syndromic causes of PNDM are due to mutations in *GATA6*, *PTF1A*, *FOXP3*, *GLIS3*, *RFX6*, *NEUROD1*,

*NEUROG3*, *HNF1B*, *PAX6*, *SLC19A2* and *WFS1* genes.

## MUTATIONS IN GATA6 GENE

This is the most common cause for pancreatic agenesis. Extra-pancreatic features are common and include structural heart defects, biliary tract and gut anomalies, and other endocrine abnormalities. Inheritance is autosomal dominant, but in most reported cases the mutations have arisen *de novo*<sup>[47]</sup>.

## PANCREATIC TRANSCRIPTION FACTOR 1, A SUBUNIT

This factor is essential for pancreatic development and function as well as cerebellar development. Other than pancreatic hypoplasia, cerebellar hypoplasia, microcephaly and respiratory distress may also develop<sup>[48]</sup>.

## FOXP3 GENE

The dysfunction of the transcription factor FOXP3 may result in X-linked-IPEX syndrome (immune dysregulation, polyendocrinopathy and enteropathy). Although autoimmune diabetes is uncommon in infancy, most of the autoimmune children may be due to *FOXP3* mutations<sup>[49]</sup>. The IPEX syndrome gene on the X chromosome, which codes for a forkhead domain-containing protein known as "scurfin", is required for immune homeostasis. These children present with intractable diarrhea with villous atrophy, exfoliative dermatitis, autoimmune hypothyroidism, hemolytic anaemia and recurrent infections. They may test positive for islet cell auto-antibodies.

## GLI SUBFAMILY OF KRUPPEL-LIKE ZINC FINGER PROTEIN-3

This transcription factor is involved in various processes in multiple tissues. These infants present with multi-system involvement like diabetes, congenital hypothyroidism, congenital glaucoma, renal cysts and dysmorphic facies<sup>[50]</sup>.

Other causes for syndromic neonatal diabetes include the following: *MX1* (motor neuron and pancreas homeobox I) mutations - neonatal diabetes with developmental delay, neurogenic bladder, sacral agenesis, and imperforate anus; *NKX2* (NK homeobox 2) mutations - neonatal diabetes with developmental delay, hypotonia, hearing impairment, cortical blindness and short stature; *RFX6* mutations - pancreatic hypoplasia, intestinal atresia, and gall bladder hypoplasia<sup>[51]</sup>. Pancreatic exocrine function is normal. Inheritance is autosomal recessive; *NEUROD1* mutations - cerebellar hypoplasia, sensorineural deafness, and visual impairment<sup>[52]</sup>. Pancreatic exocrine function is normal and the inheritance is autosomal recessive; *NEUROG3* mutations - congenital malabsorptive diarrhea and the exocrine

pancreatic function may be affected<sup>[53]</sup>. Inheritance is autosomal recessive; *HNF-1 $\beta$*  mutations - hypo plastic pancreas and renal abnormalities. The inheritance is autosomal recessive with incomplete penetrance; *PAX 6* mutations - central nervous system phenotype such as microcephaly and panhypopituitarism. The ocular phenotype includes aniridia, keratopathy, optic nerve defects, cataracts, microphthalmia and anophthalmia. Wolfram syndrome - DM with optic atrophy, diabetes insipidus and/or deafness, usually presents a little later in life although it can present in the neonatal period. Optic atrophy and diabetes may present in the first decade of life while diabetes insipidus and deafness present later in the 3<sup>rd</sup> or 4<sup>th</sup> decade<sup>[32]</sup>; *SLC19A2* (soluble carrier family 19, member 2; thiamine transporter) mutations. Recessive mutations lead to NDM, thiamine responsive megaloblastic anemia and deafness. They may have cardiac manifestations. This is also called Rogers syndrome and is inherited as an autosomal recessive disorder<sup>[54,55]</sup>; *SLC2A2* (soluble carrier family 2 member 2) mutations - Fanconi bickel syndrome. DM with hepatomegaly, glycosuria, proteinuria, hypophosphatemic rickets are the presenting features<sup>[56]</sup>. *EIF2AK3* (eukaryotic translation initiation factor 2 alpha kinase 3) mutations - Wolcott-Rallison syndrome. Infants with *EIF2AK* mutations present with neonatal diabetes, liver failure, growth retardation, epiphyseal dysplasia, developmental delay, hypothyroidism and renal failure. Higher mortality has been reported among these children. This is more common in infants born of consanguinity. Mortality is predominantly due to liver cell failure in these children<sup>[4,40]</sup>.

The course of PNDM varies by genotype. Pancreatic agenesis/hypoplasia caused by homozygous mutations in *PDX1* results in severe insulin deficiency and exocrine pancreatic insufficiency. The morbidity and mortality vary according to the co-morbid conditions of the infant. Rarely congenital hyperinsulinemia can present with neonatal diabetes with *ABCC8* mutations and is refractory to insulin therapy as in congenital lipodystrophy<sup>[57]</sup>.

## CLINICAL PRESENTATION OF NDM

Clinical features of TNDM and PNDM are indistinguishable. NDM often presents with hyperglycemia incidentally identified during evaluation for intercurrent illness or may present with keto acidosis. Rarely candida infection of the genitalia can be a presenting feature. A higher incidence of consanguinity was encountered in the study of 12 children with infantile diabetes from Chennai, India. Initial presentation as diabetic keto acidosis (DKA) was encountered in 83% of the study group. Mortality at 1-year follow-up was 16.6%<sup>[5]</sup>. Another study by Varadarajan *et al*<sup>[4]</sup> from South India revealed 67.5% of infants with ketotic onset. The median age at diagnosis was 3.75 mo. Based on the study published from South India only

32% of infants were diagnosed to have diabetes or DKA at presentation. Missed diagnosis was common in infants with diabetes (67.5%). Infantile onset diabetes present with a history of polyuria, polydipsia, irritability, vomiting, seizures, breathlessness, poor feeding, white discharge from genitals, or sticky urine. Low birth weight and monogenic diabetes were more common in those infants with onset at less than 6 mo of age in comparison with those with onset beyond 6 mo. Sixty-three percent of infants with onset in the first 6 mo of life were of low birth weight. Eighty-five percent of infants with onset less than 6 mo were identified to be monogenic in comparison to 55% if the onset was more than 6 mo. This study revealed Wolcott Rallison to be most common type of monogenic diabetes<sup>[4]</sup>. This is similar to the study by Ganesh *et al*<sup>[5]</sup> where 50% of the study group had Wolcott Rallison syndrome. Among the non-syndromic type *KCNJ11/8* was common and among the syndromic type Wolcott Rallison syndrome was common. Ten percent (4 out of 40) of infants had transient NDM with remission of hyperglycemia in the first few months and one of them relapsed at 9.7 years of age. Children with Wolcott Rallison syndrome had a higher mortality than any other group. Hepatic failure was the most common cause of death. Co-morbid states encountered in infants with diabetes include developmental delay, seizures, hepatic involvement, hypothyroidism, optic atrophy, hepatomegaly, short stature and rickets. The mean insulin requirement was 1.19 units/kg per day in those with onset less than 6 mo or 1.4 units/kg per day in those with onset more than 6 mo. Among those children with *KCNJ11* or *ABCC8* mutation the response to oral sulphonylurea is excellent and better metabolic control has been documented even during follow-up. Other than glibenclamide, glyburide has also been tried in children with *KCNJ11* or *ABCC8* mutation. Relapsing NDM in older children do respond well to sulphonylurea without need for insulin therapy. Despite the advances of diagnosis and management of IODM, day-to-day problems exist in the management in developing countries. One of the problems of concern is the assessment of glycemic control among infants who are on 2-3 hourly breast feeds or demand feeds. Difficulty in dispensing very low doses of insulin is a problem in developing countries. Delivering insulin less than 1 unit is difficult as there are no diluents available in developing countries. Dilution with normal saline have been used in such situations but the evidence for the same to be effective is lacking. Currently available insulin pens to dispense 0.5 units may be useful in such infants. The psychological trauma to the family members and lack of adequate support in the community through a structured diabetic care team makes day-to-day management of diabetes difficult in these infants. Frequent hospitalization, glycemic fluctuations, poor weight gain and co-morbid conditions were other problems reported in studies from South India<sup>[4]</sup>. Literature reveals continuous subcutaneous infusion of insulin as a useful intervention, although

affordability and cost are limiting factors in developing countries. Limited availability of genetic studies in India may cause delay in the diagnosis of monogenic diabetes. Reported mortality in infantile onset diabetes is very high in developing countries. The mortality at 1-year follow-up was 16%<sup>[5]</sup>. However, another study of 40 infants with diabetes from South India had revealed a mortality of 32.5% over a 12-year period<sup>[4]</sup>. Cerebral edema, sepsis, acute respiratory distress syndrome, disseminated intravascular coagulation, hypoglycemia, refractory cardiac failure, septic shock, renal failure and hepatic failure were the causes of mortality in infantile onset diabetes.

Comparing the literature about IODM in developed countries, the following needs to be emphasized. TNDM is reported to constitute 40%-60% of IODM cases in Western literature while it is less than 10% from developing countries. The infrequent sequencing for 6q mutations may be a possible explanation. Those with onset between 6 to 12 mo were commonly identified to have *INS* gene mutation in comparison to the *EIF2AK* mutation in developing countries, and a higher rate of consanguinity may be a contributory factor. Comprehensive genetic testing has identified genetic cause in more than 80% of IODM cases in developed countries, while it is much lower in developing countries. Nearly 60%-80% of IODM cases present with DKA in developing countries while no such data are available from developed countries. Developmental delay and neuropsychological dysfunction are common in children with IODM from developed countries while developmental delay and hepatosplenomegaly have been reported from developing countries. Insulin pumps have been used for insulin requiring mutations in developed countries while they have been managed with conventional injections in developing countries like India. Overall mortality in IODM was reported to be 33% in developing countries while no such data on mortality in IODM are available from developed countries.

## DIAGNOSTIC APPROACH TO INFANTILE ONSET NEONATAL DIABETES

A low C peptide level and high HBA1c level are supporting lab parameters to confirm infantile onset diabetes from stress induced hyperglycemia in infants. Initial evaluation should include a search for syndromic features. Associated features like hypothyroidism, elevated liver enzymes, skeletal dysgenesis, pancreatic agenesis, enteropathy, developmental delay, anemia, umbilical hernia and macroglossia should be looked for (Table 1). All infants with onset of diabetes at less than one year of age need to undergo genetic evaluation at the earliest for monogenic diabetes. Older children of any age with infantile onset diabetes can undergo genetic work-up as therapy with sulphonylurea at a later age is still useful for good glycemic control and management

**Table 1 Diagnostic clues for type of mutation in infantile onset diabetes mellitus**

Associated features	Diagnostic possibility of mutation
Umbilical hernia, macroglossia	6q 24
Developmental delay	<i>KCNJ11</i> , <i>ABCC8</i> , <i>EIF2AK3</i>
Microcephaly	<i>PTF 1A</i>
Hypothyroidism	<i>EIF2AK3</i> , <i>GLIS 3</i> , <i>FOXP3</i>
Diarrhea, eczema	<i>IPEX</i>
Anemia	<i>EIF2AK3</i> , <i>SLC19A2</i>
Hepatomegaly with liver dysfunction	<i>EIF2AK3</i>
Cerebellar hypoplasia	<i>PTF1A</i> , <i>NEUROD 1</i>
Pancreatic hypoplasia	<i>RFX 6</i> , <i>HNFB1</i> , <i>PTF1A</i> , <i>GATA6</i>
Ocular manifestations	<i>PAX 6</i>
Rickets, round facies, mild hyperglycemia	<i>SLC2A2</i>
No syndromic features	<i>KCNJ11</i> , <i>ABCC8</i> , <i>INS</i>
Renal abnormalities	<i>GLIS3</i> , <i>HNFB1</i>
Hirsutism, failure to thrive	Insulin resistance syndromes

*KCNJ11*: Potassium channel subfamily J member 11; *ABCC8*: ATP binding cassette transporter subfamily C, member 8; *EIF2AK3*: Eukaryotic translation initiation factor 2 alpha kinase 3; *PTF*: Pancreatic transcription factor; *GLIS3*: GLI subfamily of Kruppel-like zinc finger protein-3; *SLC2A2*: Solute carrier 2 family 2 gene; *INS*: Insulin.

of other comorbid factors. However, long-term insulin therapy may reduce the available beta cell mass and they may need additional glucose reducing drugs. Hence, an earlier genetic confirmation or therapy with sulphonylurea is warranted. Comprehensive genetic screening has been found to be more useful for early diagnosis than the conventional genetic screening. Conventional genetic tests analyze few genes based on the clinical features. With improved sequencing methods simultaneous analysis of multiple genes is possible. The genetic result predicts the best diabetes treatment and development of associated features. Evaluation with auto-immune antibodies may be warranted in infants with onset of diabetes in late infancy as the chances of type 1 diabetes presenting in late infancy has been reported in the literature.

## Management of IODM

Adequate hydration of infants with acute presentation in DKA is essential. Infants with DKA may need much more fluids than the management of older children. Associated predisposing factors like sepsis or bronchopneumonia need to be treated for early control of hyperglycemia. Infusions of short-acting insulin in DKA and subcutaneous doses of insulin are the therapy of choice until evaluation for monogenic diabetes. These infants and toddlers may be very sensitive to small doses of insulin and careful watch for hypoglycemia is a must. Avoiding hypoglycemia is essential in these infants as sequelae of hypoglycemia on the developing brain leads to increased morbidity. Short-acting and rapid-acting insulin may sometimes cause hypoglycemia that is difficult to control in infants. Intermediate-acting insulin is preferred to be given as once or twice a day therapy<sup>[4]</sup>. Initial insulin dose for stabilization may

range from 0.35 units/kg per day to 3 units/kg per day. Insulin pumps have been used successfully in developed countries<sup>[58,59]</sup>, but the initial cost and subsequent maintenance are major issues in using insulin pumps in infants from developing countries like India. If genetic reports suggest mutations in *KCNJ11* or *ABCC8* mutations which are responsive to sulphonylurea, transfer to oral drugs should be undertaken. Earlier identification of sulphonylurea responsiveness (*KCNJ11* and *ABCC8* mutations) is essential as the insulin therapy will only achieve glycemic control. Other systemic manifestations like seizures, muscular involvement, and developmental delay do not respond to insulin therapy. Earlier institution of sulphonylurea is essential to prevent worsening of these co-morbid factors in IODM. Following genetic confirmation, insulin can be switched slowly by outpatient protocols or by rapid inpatient protocols<sup>[21,60-66]</sup>. The initial median dose for sulphonylurea used to treat patients with  $K_{ATP}$  channel mutations is 0.45 mg/kg per day (range, 0.05-1.75 mg/kg per day). The switch over from insulin therapy to sulphonylurea may take more than 6 wk as some infants need very high doses up to 2 mg/kg per day of sulphonylurea<sup>[4]</sup>. In developing countries like India awaiting genetic reports for sulphonylurea therapy may cause a delay in specific treatment for co-morbid conditions. Studies have been undertaken to assess the risk and benefit of sulphonylurea therapy prior to genetic confirmation. In view of the potential benefit in the neurodevelopmental outcome and glycemic control, one may consider empirical inpatient trial with sulphonylurea if the genetic results are likely to be delayed. However, further studies with a large number of infants are warranted to decide on empirical sulphonylurea therapy<sup>[67]</sup>. Age at initiation of sulphonylurea showed a linear correlation with the dose required at follow-up. Indeed, all patients required additional glucose lowering medications along with sulphonylurea, if drug therapy was started at the age of 13 years or older<sup>[68]</sup>. Short-term studies on sulphonylurea therapy in children have been found to be safe without major side effects. Diarrheal episodes and rarely discolouration of teeth have been reported with sulphonylurea. Diarrhea usually disappears with therapy. None of these side effects will affect the continuation of therapy<sup>[63,69]</sup>. It is imperative to confirm the genetic mutation to decide treatment, to assess prognosis and anticipate the long term-outcome. They need to be followed even after remission as relapse has been reported in early adolescence or adults. The relapse responds well to sulphonylurea therapy.

These children need to be followed for glycemic control, growth pattern, developmental delay, seizures, pancreatic exocrine function, hypothyroidism and other comorbid states depending on the type of mutation. Periodic monitoring for long-term complications is mandatory. Presently there are no guidelines for long-term follow-up for diabetes related complications in these children with IODM, as long-term follow-

up studies are lacking. Evaluation of the parental diabetic status and the genetic mutations will help to plan counseling regarding the future conceptions in the family. A neonatal diabetes registry has been established in India where facilities are available to sequence *KCNJ11*, *ABCC8*, *insulin* genes and other syndromic mutations.

## CONCLUSION

DKA is the most common presentation of IODM (67%-83%). Low birth weight is common in infants less than 6 mo of age at presentation. Sixty-seven point five percent of IODM cases had a missed diagnosis at presentation. Monogenic diabetes is the most common cause of infantile onset diabetes. *KCNJ11/ABCC8* and *EIF2AK* mutations are the commonly reported non-syndromic and syndromic types, respectively. TNDM was noted in 10% of all infantile onset diabetics in South India. Developmental delay and seizures are major co-morbid factors in IODM. Glycemic control assessment among breast-fed infants and dispensing very small doses of insulin are difficult in IODM in developing countries. Management is by once or twice a day injections of intermediate-acting subcutaneous insulin, and continuous subcutaneous insulin infusions if feasible. Potassium channel mutations (*KCNJ11* and *ABCC8*) are sulphonylurea responsive and infants may need higher doses up to 2 mg/kg per day. Reported mortality during follow-up of IODM is very high (33%) in India.

## REFERENCES

- 1 **Aguilar-Bryan L**, Bryan J. Neonatal diabetes mellitus. *Endocr Rev* 2008; **29**: 265-291 [PMID: 18436707 DOI: 10.1210/er.2007-0029]
- 2 **Mohamadi A**, Clark LM, Lipkin PH, Mahone EM, Wodka EL, Plotnick LP. Medical and developmental impact of transition from subcutaneous insulin to oral glyburide in a 15-yr-old boy with neonatal diabetes mellitus and intermediate DEND syndrome: extending the age of *KCNJ11* mutation testing in neonatal DM. *Pediatr Diabetes* 2010; **11**: 203-207 [PMID: 19686306 DOI: 10.1111/j.1399-5448.2009.00548.x]
- 3 **Polak M**, Cavé H. Neonatal diabetes mellitus: a disease linked to multiple mechanisms. *Orphanet J Rare Dis* 2007; **2**: 12 [PMID: 17349054 DOI: 10.1186/1750-1172-2-12]
- 4 **Varadarajan P**, Sangaralingam T, Senniappan S, Jahnvi S, Radha V, Mohan V. Clinical profile and outcome of infantile onset diabetes mellitus in southern India. *Indian Pediatr* 2013; **50**: 759-763 [PMID: 23502672]
- 5 **Ganesh R**, Arvindkumar R, Vasanthi T. Infantile-onset diabetes mellitus: a 1-year follow-up study. *Clin Pediatr (Phila)* 2009; **48**: 271-274 [PMID: 18836058 DOI: 10.1177/0009922808324950]
- 6 **De Franco E**, Flanagan SE, Houghton JA, Lango Allen H, Mackay DJ, Temple IK, Ellard S, Hattersley AT. The effect of early, comprehensive genomic testing on clinical care in neonatal diabetes: an international cohort study. *Lancet* 2015; **386**: 957-963 [PMID: 26231457]
- 7 **Holzinger A**, Bonfig W, Kusser B, Eggermann T, Müller H, Munch HG. Use of long-term microdialysis subcutaneous glucose monitoring in the management of neonatal diabetes. A first case report. *Biol Neonate* 2006; **89**: 88-91 [PMID: 16166771 DOI: 10.1159/000088349]
- 8 **von Mühlendahl KE**, Herkenhoff H. Long-term course of



- neonatal diabetes. *N Engl J Med* 1995; **333**: 704-708 [PMID: 7637748]
- 9 **Cavé H**, Polak M, Drunat S, Denamur E, Czernichow P. Refinement of the 6q chromosomal region implicated in transient neonatal diabetes. *Diabetes* 2000; **49**: 108-113 [PMID: 10615957]
- 10 **Temple IK**, Gardner RJ, Robinson DO, Kibirige MS, Ferguson AW, Baum JD, Barber JC, James RS, Shield JP. Further evidence for an imprinted gene for neonatal diabetes localised to chromosome 6q22-q23. *Hum Mol Genet* 1996; **5**: 1117-1121 [PMID: 8842729]
- 11 **Gardner RJ**, Mackay DJ, Mungall AJ, Polychronakos C, Siebert R, Shield JP, Temple IK, Robinson DO. An imprinted locus associated with transient neonatal diabetes mellitus. *Hum Mol Genet* 2000; **9**: 589-596 [PMID: 10699182]
- 12 **Temple IK**, Mackay D, Docherty LE. 6q24-Related Transient Neonatal Diabetes Mellitus. In: Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LJH, Bird TD, Fong CT, Mefford HC, Smith RJH, Stephens K, editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2015. 2005 Oct 10 [updated 2015 Jan 15] [PMID: 20301706]
- 13 **Flanagan S**. Transient neonatal diabetes. Diapedia. Available from: URL: <http://www.diapedia.org/41040851198/rev/23>
- 14 **Gloyn AL**, Reimann F, Girard C, Edghill EL, Proks P, Pearson ER, Temple IK, Mackay DJ, Shield JP, Freedenberg D, Noyes K, Ellard S, Ashcroft FM, Gribble FM, Hattersley AT. Relapsing diabetes can result from moderately activating mutations in KCNJ11. *Hum Mol Genet* 2005; **14**: 925-934 [PMID: 15718250]
- 15 **Babenko AP**, Polak M, Cavé H, Busiah K, Czernichow P, Scharfmann R, Bryan J, Aguilar-Bryan L, Vaxillaire M, Froguel P. Activating mutations in the ABCC8 gene in neonatal diabetes mellitus. *N Engl J Med* 2006; **355**: 456-466 [PMID: 16885549]
- 16 **Proks P**, Arnold AL, Bruining J, Girard C, Flanagan SE, Larkin B, Colclough K, Hattersley AT, Ashcroft FM, Ellard S. A heterozygous activating mutation in the sulphonylurea receptor SUR1 (ABCC8) causes neonatal diabetes. *Hum Mol Genet* 2006; **15**: 1793-1800 [PMID: 16613899]
- 17 **Flanagan SE**, Patch AM, Mackay DJ, Edghill EL, Gloyn AL, Robinson D, Shield JP, Temple K, Ellard S, Hattersley AT. Mutations in ATP-sensitive K<sup>+</sup> channel genes cause transient neonatal diabetes and permanent diabetes in childhood or adulthood. *Diabetes* 2007; **56**: 1930-1937 [PMID: 17446535]
- 18 **Schimmel U**. Long-standing sulphonylurea therapy after pubertal relapse of neonatal diabetes in a case of uniparental paternal isodisomy of chromosome 6. *Diabetes Care* 2009; **32**: e9 [PMID: 19114626]
- 19 **Poovazhagi V**, Thangavelu S. Relapsing Transient Neonatal Diabetes Mellitus due to ABCC8 Mutation. *J Mol Genet Med* 2014; **8**: 136 [DOI: 10.4172/1747-0862.1000136]
- 20 **Yorifuji T**, Hashimoto Y, Kawakita R, Hosokawa Y, Fujimaru R, Hatake K, Tamagawa N, Nakajima H, Fujii M. Relapsing 6q24-related transient neonatal diabetes mellitus successfully treated with a dipeptidyl peptidase-4 inhibitor: a case report. *Pediatr Diabetes* 2014; **15**: 606-610 [PMID: 24552466 DOI: 10.1111/pedi.12123]
- 21 **Pearson ER**, Flechtner I, Njolstad PR, Malecki MT, Flanagan SE, Larkin B, Ashcroft FM, Klimes I, Codner E, Iotova V, Slingerland AS, Shield J, Robert JJ, Holst JJ, Clark PM, Ellard S, Søvik O, Polak M, Hattersley AT. Switching from insulin to oral sulphonylureas in patients with diabetes due to Kir6.2 mutations. *N Engl J Med* 2006; **355**: 467-477 [PMID: 16885550]
- 22 **Nair VV**, Chapla A, Arulappan N, Thomas N. Molecular diagnosis of maturity onset diabetes of the young in India. *Indian J Endocrinol Metab* 2013; **17**: 430-441 [PMID: 23869298]
- 23 **Kochhar IP**, Kulkarni KP. Transient Neonatal Diabetes due to Kcnj11 Mutation. *Indian Pediatr* 2010; **47**: 359-360 [PMID: 20431170]
- 24 **Benon PS**, Khatwa UA. Diabetes mellitus in newborns and infants. *Indian J Pediatr* 2000; **67**: 443-448 [PMID: 10932965]
- 25 **Batra CM**, Gupta N, Atwal G, Gupta V. Transient neonatal diabetes due to activating mutation in the ABCC8 gene encoding SUR1. *Indian J Pediatr* 2009; **76**: 1169-1172 [PMID: 20092027]
- 26 **Rais N**, Joshi M. Transient neonatal diabetes mellitus. *Indian J Pediatr* 1988; **55**: 979-982 [PMID: 3235149]
- 27 **Kumar SS**, Premalatha G, Mohan V. Infantile Type 1 Diabetes Mellitus (Onset Less than One Year of Age) - A Report of Eight Patients. *Int J Diab Dev Ctries* 2002; **22**: 103-106
- 28 **Merchant R**, Irani A, Nagar P. Transient diabetes mellitus in early infancy. *Indian Pediatr* 1985; **22**: 529-532 [PMID: 3914466]
- 29 **Seth A**, Sharda S, Narula MK, Aneja S, Taluja V. Diabetes mellitus in an infant. *Indian J Pediatr* 2004; **71**: 947 [PMID: 15531847 DOI: 10.1007/BF02830846]
- 30 **Jali MV**, Patil VD, Jali SM, Gowda S, Kambar S. Type 1 diabetes mellitus with ketoacidosis in infancy. *Indian J Pediatr* 2009; **76**: 424-426 [PMID: 19205630]
- 31 **Flanagan S**. Permanent neonatal diabetes. Diapedia 2014. Available from: URL: <http://www.diapedia.org/41040851200/rev/31>
- 32 **De León DD**, Stanley CA. Permanent Neonatal Diabetes Mellitus. In: Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LJH, Bird TD, Fong CT, Mefford HC, Smith RJH, Stephens K, editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2015. 2008 Feb 8 [updated 2014 Jan 23] [PMID: 20301620]
- 33 **Zhang M**, Chen X, Shen S, Li T, Chen L, Hu M, Cao L, Cheng R, Zhao Z, Luo F. Sulphonylurea in the treatment of neonatal diabetes mellitus children with heterogeneous genetic backgrounds. *J Pediatr Endocrinol Metab* 2015; **28**: 877-884 [PMID: 25781672 DOI: 10.1515/jpem-2014-0429]
- 34 **Hattersley AH**, Ashcroft FM. Activating Mutations in Kir6.2 and Neonatal Diabetes. *Diabetes* 2005; **54**: 2503-2513 [DOI: 10.2337/diabetes.54.9.2503]
- 35 **Singh P**, Rao SC, Parikh R. Neonatal diabetes with intractable epilepsy: DEND syndrome. *Indian J Pediatr* 2014; **81**: 1387-1388 [PMID: 24912436]
- 36 **Letha S**, Mammen D, Valampampil JJ. Permanent neonatal diabetes due to KCNJ11 gene mutation. *Indian J Pediatr* 2007; **74**: 947-949 [PMID: 17978456]
- 37 **Jain V**, Flanagan SE, Ellard S. Permanent neonatal diabetes caused by a novel mutation. *Indian Pediatr* 2012; **49**: 486-488 [PMID: 22796691]
- 38 **Joshi R**, Phatarpekar A. Neonatal diabetes mellitus due to L233F mutation in the KCNJ11 gene. *World J Pediatr* 2011; **7**: 371-372 [PMID: 21210267 DOI: 10.1007/s12519-011-0254-z]
- 39 **Slingerland AS**, Hurkx W, Noordam K, Flanagan SE, Jukema JW, Meiners LC, Bruining GJ, Hattersley AT, Hadders-Algra M. Sulphonylurea therapy improves cognition in a patient with the V59M KCNJ11 mutation. *Diabet Med* 2008; **25**: 277-281 [PMID: 18307455]
- 40 **Jahnavi S**, Poovazhagi V, Mohan V, Bodhini D, Raghupathy P, Amutha A, Suresh Kumar P, Adhikari P, Shriram M, Kaur T, Das AK, Molnes J, Njolstad PR, Unnikrishnan R, Radha V. Clinical and molecular characterization of neonatal diabetes and monogenic syndromic diabetes in Asian Indian children. *Clin Genet* 2013; **83**: 439-445 [PMID: 22831748]
- 41 **Støy J**, Edghill EL, Flanagan SE, Ye H, Paz VP, Pluzhnikov A, Below JE, Hayes MG, Cox NJ, Lipkind GM, Lipton RB, Greeley SA, Patch AM, Ellard S, Steiner DF, Hattersley AT, Philipson LH, Bell GI. Insulin gene mutations as a cause of permanent neonatal diabetes. *Proc Natl Acad Sci USA* 2007; **104**: 15040-15044 [PMID: 17855560]
- 42 **Garin I**, Edghill EL, Akerman I, Rubio-Cabezas O, Rica I, Locke JM, Maestro MA, Alshaikh A, Bundak R, del Castillo G, Deeb A, Deiss D, Fernandez JM, Godbole K, Hussain K, O'Connell M, Klupa T, Kolouskova S, Mohsin F, Perlman K, Sumnik Z, Rial JM, Ugarte E, Vasanthi T, Johnstone K, Flanagan SE, Martínez R, Castaño C, Patch AM, Fernández-Rebollo E, Raile K, Morgan N, Harries LW, Castaño L, Ellard S, Ferrer J, Perez de Naclores G, Hattersley AT. Recessive mutations in the INS gene result in neonatal diabetes through reduced insulin biosynthesis. *Proc Natl Acad Sci USA* 2010; **107**: 3105-3110 [PMID: 20133622 DOI:

- 10.1073/pnas.0910533107]
- 43 **Ahamed A**, Unnikrishnan AG, Pendsey SS, Nampoothiri S, Bhavani N, Praveen VP, Kumar H, Jayakumar RV, Nair V, Ellard S, Edghill EL. Permanent neonatal diabetes mellitus due to a C96Y heterozygous mutation in the insulin gene. A case report. *JOP* 2008; **9**: 715-718 [PMID: 18981553]
  - 44 **Turkkahraman D**, Bircan I, Tribble ND, Akçurin S, Ellard S, Gloyne AL. Permanent neonatal diabetes mellitus caused by a novel homozygous (T168A) glucokinase (GCK) mutation: initial response to oral sulphonylurea therapy. *J Pediatr* 2008; **153**: 122-126 [PMID: 18571549]
  - 45 **Bennett K**, James C, Mutair A, Al-Shaikh H, Sinani A, Hussain K. Four novel cases of permanent neonatal diabetes mellitus caused by homozygous mutations in the glucokinase gene. *Pediatr Diabetes* 2011; **12**: 192-196 [PMID: 21518409]
  - 46 **Stoffers DA**, Zinkin NT, Stanojevic V, Clarke WL, Habener JF. Pancreatic agenesis attributable to a single nucleotide deletion in the human IPF1 gene coding sequence. *Nat Genet* 1997; **15**: 106-110 [PMID: 8988180]
  - 47 **De Franco E**, Shaw-Smith C, Flanagan SE, Shepherd MH, Hattersley AT, Ellard S. GATA6 mutations cause a broad phenotypic spectrum of diabetes from pancreatic agenesis to adult-onset diabetes without exocrine insufficiency. *Diabetes* 2013; **62**: 993-997 [PMID: 23223019 DOI: 10.2337/db12-0885]
  - 48 **Hoveyda N**, Shield JP, Garrett C, Chong WK, Beardsall K, Bentsi-Enchill E, Mallya H, Thompson MH. Neonatal diabetes mellitus and cerebellar hypoplasia/agenesis: report of a new recessive syndrome. *J Med Genet* 1999; **36**: 700-704 [PMID: 10507728]
  - 49 **Rubio-Cabezas O**, Minton JA, Caswell R, Shield JP, Deiss D, Sumnik Z, Cayssials A, Herr M, Loew A, Lewis V, Ellard S, Hattersley AT. Clinical heterogeneity in patients with FOXP3 mutations presenting with permanent neonatal diabetes. *Diabetes Care* 2009; **32**: 111-116 [PMID: 18931102]
  - 50 **Senée V**, Chelala C, Duchatelet S, Feng D, Blanc H, Cossec JC, Charon C, Nicolino M, Boileau P, Cavener DR, Bougnères P, Taha D, Julier C. Mutations in GLIS3 are responsible for a rare syndrome with neonatal diabetes mellitus and congenital hypothyroidism. *Nat Genet* 2006; **38**: 682-687 [PMID: 16715098]
  - 51 **Concepcion JP**, Reh CS, Daniels M, Liu X, Paz VP, Ye H, Highland HM, Hanis CL, Greeley SA. Neonatal diabetes, gallbladder agenesis, duodenal atresia, and intestinal malrotation caused by a novel homozygous mutation in RFX6. *Pediatr Diabetes* 2014; **15**: 67-72 [PMID: 23914949]
  - 52 **Rubio-Cabezas O**, Minton JA, Kantor I, Williams D, Ellard S, Hattersley AT. Homozygous mutations in NEUROD1 are responsible for a novel syndrome of permanent neonatal diabetes and neurological abnormalities. *Diabetes* 2010; **59**: 2326-2331 [PMID: 20573748 DOI: 10.2337/db10-0011]
  - 53 **Pinney SE**, Oliver-Krasinski J, Ernst L, Hughes N, Patel P, Stoffers DA, Russo P, De León DD. Neonatal diabetes and congenital malabsorptive diarrhea attributable to a novel mutation in the human neurogenin-3 gene coding sequence. *J Clin Endocrinol Metab* 2011; **96**: 1960-1965 [PMID: 21490072]
  - 54 **Ganesh R**, Ezhilarasi S, Vasanthi T, Gowrishankar K, Rajajee S. Thiamine responsive megaloblastic anemia syndrome. *Indian J Pediatr* 2009; **76**: 313-314 [PMID: 19347672]
  - 55 **Shaw-Smith C**, Flanagan SE, Patch AM, Grulich-Henn J, Habeb AM, Hussain K, Pomahacova R, Matyka K, Abdullah M, Hattersley AT, Ellard S. Recessive SLC19A2 mutations are a cause of neonatal diabetes mellitus in thiamine-responsive megaloblastic anaemia. *Pediatr Diabetes* 2012; **13**: 314-321 [PMID: 22369132]
  - 56 **Poovazhagi V**, Sridhurga U, Prabha S, Sujatha J. A Novel Mutation in the GLUT2 gene – Case report of Fanconi-Bickel Syndrome in a Female Indian Patient. *J Hypo Hyperglycemia* 2015; **2**: 1 [DOI: 10.4172/2327-4700.1000106]
  - 57 **Poovazhagi V**, Shanthi S, Jahnvi S, Radha V, Mohan V, Berardinelli Seip congenital lipodystrophy presenting with neonatal diabetes mellitus due to a mutation in the AGPAT2 gene. *Int J Diabetes Dev Ctries* 2013; **33**: 129-129 [DOI: 10.1007/s13410-012-0099-6]
  - 58 **Tubiana-Rufi N**. Insulin pump therapy in neonatal diabetes. *Endocr Dev* 2007; **12**: 67-74 [PMID: 17923770]
  - 59 **Beardsall K**, Pesterfield CL, Acerini CL. Neonatal diabetes and insulin pump therapy. *Arch Dis Child Fetal Neonatal Ed* 2011; **96**: F223-F224 [PMID: 21115555]
  - 60 **Hattersley AT**. Transferring patients who have a mutation in KCJ11 or ABCC8. Available from: URL: <http://www.diabetesgenes.org/content/transferring-patients-who-have-mutation-kcnj11-or-abcc8>
  - 61 **Poovazhagi V**, Muralidharan PS, Parivathini S. Neonatal diabetes with KIR 6.2 mutation on glibenclamide therapy. *Pediatric Oncall* [serial online] 2012 [cited 2012 Apr 1]; **9**. Art #23. Available from: URL: <http://www.pediatriconcall.com/Journal/Article/FullText.aspx?artid=473&type=J&tid=&imgid=&reportid=52&tbltype=>
  - 62 **Codner E**, Flanagan S, Ellard S, García H, Hattersley AT. High-dose glibenclamide can replace insulin therapy despite transitory diarrhea in early-onset diabetes caused by a novel R201L Kir6.2 mutation. *Diabetes Care* 2005; **28**: 758-759 [PMID: 15735229]
  - 63 **Sagen JV**, Raeder H, Hathout E, Shehadeh N, Gudmundsson K, Baevre H, Abuelo D, Phornphutkul C, Molnes J, Bell GI, Gloyne AL, Hattersley AT, Molven A, Søvik O, Njølstad PR. Permanent neonatal diabetes due to mutations in KCNJ11 encoding Kir6.2: patient characteristics and initial response to sulphonylurea therapy. *Diabetes* 2004; **53**: 2713-2718 [PMID: 15448106]
  - 64 **Slingerland AS**, Nuboe R, Hadders-Algra M, Hattersley AT, Bruining GJ. Improved motor development and good long-term glycaemic control with sulphonylurea treatment in a patient with the syndrome of intermediate developmental delay, early-onset generalised epilepsy and neonatal diabetes associated with the V59M mutation in the KCNJ11 gene. *Diabetologia* 2006; **49**: 2559-2563 [PMID: 17047922]
  - 65 **Zung A**, Glaser B, Nimri R, Zadik Z. Glibenclamide treatment in permanent neonatal diabetes mellitus due to an activating mutation in Kir6.2. *J Clin Endocrinol Metab* 2004; **89**: 5504-5507 [PMID: 15531505]
  - 66 **Klupa T**, Edghill EL, Nazim J, Sieradzki J, Ellard S, Hattersley AT, Malecki MT. The identification of a R201H mutation in KCNJ11, which encodes Kir6.2, and successful transfer to sustained-release sulphonylurea therapy in a subject with neonatal diabetes: evidence for heterogeneity of beta cell function among carriers of the R201H mutation. *Diabetologia* 2005; **48**: 1029-1031 [PMID: 15838686]
  - 67 **Carmody D**, Bell CD, Hwang JL, Dickens JT, Sima DI, Felipe DL, Zimmer CA, Davis AO, Kotlyarevska K, Naylor RN, Philipson LH, Greeley SA. Sulphonylurea treatment before genetic testing in neonatal diabetes: pros and cons. *J Clin Endocrinol Metab* 2014; **99**: E2709-E2714 [PMID: 25238204 DOI: 10.1210/jc.2014-2494]
  - 68 **Thurber BW**, Carmody D, Tadie EC, Pastore AN, Dickens JT, Wroblewski KE, Naylor RN, Philipson LH, Greeley SA. Age at the time of sulphonylurea initiation influences treatment outcomes in KCNJ11-related neonatal diabetes. *Diabetologia* 2015; **58**: 1430-1435 [PMID: 25877689 DOI: 10.1007/s00125-015-3593-9]
  - 69 **Kumaraguru J**, Flanagan SE, Greeley SA, Nuboe R, Stoy J, Philipson LH, Hattersley AT, Rubio-Cabezas O. Tooth discoloration in patients with neonatal diabetes after transfer onto glibenclamide: a previously unreported side effect. *Diabetes Care* 2009; **32**: 1428-1430 [PMID: 19435956 DOI: 10.2337/dc09-0280]

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## Novel nutraceutical therapies for the treatment of metabolic syndrome

Esperanza Martínez-Abundis, Miriam Mendez-del Villar, Karina G Pérez-Rubio, Laura Y Zuñiga, Marisol Cortez-Navarrete, Alejandra Ramírez-Rodriguez, Manuel González-Ortiz

Esperanza Martínez-Abundis, Miriam Mendez-del Villar, Karina G Pérez-Rubio, Laura Y Zuñiga, Marisol Cortez-Navarrete, Alejandra Ramírez-Rodriguez, Manuel González-Ortiz, Institute of Experimental and Clinical Therapeutics, Department of Physiology, Health Sciences University Center, University of Guadalajara, Guadalajara 44340, Jalisco, Mexico

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**Correspondence to:** Manuel González-Ortiz, MD, MSc, PhD, Institute of Experimental and Clinical Therapeutics, Department of Physiology, Health Sciences University Center, University of Guadalajara, Montes Urales 1409, Colonia Independencia, Guadalajara 44340, Jalisco, Mexico. [uiec@prodigy.net.mx](mailto:uiec@prodigy.net.mx)  
 Telephone: +52-33-38267022  
 Fax: +52-33-36161218

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### Abstract

Nutraceutical therapies such as berberine, bitter melon,

*Gymnema sylvestre*, *Irvingia gabonensis*, resveratrol and ursolic acid have been shown to help control metabolic syndrome (MetS). The effect of berberine on glucose and lipid metabolism, hypertension, obesity and MetS has been evaluated in animal models and humans. Most clinical trials involving bitter melon have been conducted to evaluate its effect on glucose metabolism; nevertheless, some studies have reported favorable effects on lipids and blood pressure although there is little information about its effect on body weight. *Gymnema sylvestre* helps to decrease body weight and blood sugar levels; however, there is limited information on dyslipidemia and hypertension. Clinical trials of *Irvingia gabonensis* have shown important effects decreasing glucose and cholesterol concentrations as well decreasing body weight. Resveratrol acts through different mechanisms to decrease blood pressure, lipids, glucose and weight, showing its effects on the population with MetS. Finally, there is evidence of positive effects with ursolic acid in *in vitro* and *in vivo* studies on glucose and lipid metabolism and on body weight and visceral fat. Therefore, a review of the beneficial effects and limitations of the above-mentioned nutraceutical therapies is presented.

**Key words:** Nutraceuticals; Metabolic syndrome; Berberine; Bitter melon; *Gymnema sylvestre*; *Irvingia gabonensis*; Resveratrol; Ursolic acid

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**Core tip:** Metabolic syndrome (MetS) is a cluster of endocrine problems including obesity, dysglycemia, dyslipidemia, and hypertension. Unfortunately, there is no unique treatment to control it. Nutraceutical therapies such as berberine, bitter melon, *Gymnema sylvestre*, *Irvingia gabonensis*, resveratrol and ursolic acid have demonstrated some improvement in anthropometric parameters and cardiometabolic risk factors and could

be considered as treatment for patients with MetS. This review attempts to demonstrate the beneficial effects and limitations of some of these novel nutraceutical therapies.

Martínez-Abundis E, Méndez-del Villar M, Pérez-Rubio KG, Zuñiga LY, Cortez-Navarrete M, Ramírez-Rodríguez A, González-Ortiz M. Novel nutraceutical therapies for the treatment of metabolic syndrome. *World J Diabetes* 2016; 7(7): 142-152 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v7/i7/142.htm> DOI: <http://dx.doi.org/10.4239/wjd.v7.i7.142>

## INTRODUCTION

Metabolic syndrome (MetS) is a cluster of endocrine disturbances including typically obesity, dysglycemia, dyslipidemia, and hypertension, predisposing individuals to increased risk for atherosclerosis, cardiovascular events, and eventually type 2 diabetes mellitus (T2DM)<sup>[1]</sup>. However, a number of other parameters that appear to be related to MetS, including non-alcoholic fatty liver disease, should be evaluated in some specific cases to help determine the risk of complications<sup>[2,3]</sup>. Prevalence of MetS is increasing significantly and is becoming a worldwide health problem<sup>[4]</sup>. Unfortunately, there is no a single treatment to control MetS; frequently, the option is to treat each component separately. Therefore, any substance that helps to control all the characteristic disturbances of MetS must be considered and studied in depth<sup>[5]</sup>. Nutraceutical therapies such as berberine, bitter melon, *Gymnema sylvestre* (*G. sylvestre*), *Irvingia gabonensis* (*I. gabonensis*), resveratrol and ursolic acid, which are currently being studied in our Research Institute, among many therapies, have demonstrated to improve some anthropometric parameters and cardiometabolic risk factors. In this regard, they could be considered as treatment for patients with MetS. The aim of this review is to show the beneficial effects and limitations of some of these novel nutraceutical therapies.

## BERBERINE

Berberine is an isoquinoline quaternary alkaloid (or a 5,6 dihydrodibenzo[*a,g*]quinolizinium derivative) isolated from many medicinal plants such as *Hydrastis canadensis*, *Berberis aristata*, *Coptis chinensis*, *Coptis rhizome*, *Coptis japonica*, *Phellodendron amurense* and *Phellodendron chinense schneid*<sup>[6]</sup>. Berberine is traditionally used for its supposed antimicrobial effects and as treatment for diabetes in traditional Chinese, Indian and Middle Eastern folk medicine<sup>[7]</sup> and has definite potential as a drug included in a wide spectrum of clinical applications.

During approximately 500 A.D., Hongjing Tao recorded the anti-diabetes activity of *Rhizoma coptidis* for the first time in a book entitled "Note of Elite

Physicians". In 1988, the hypoglycemic effect of berberine was revealed when berberine was used to treat diarrhea in diabetic patients in China. Since that time, many physicians in China have used berberine as an anti-hyperglycemic agent. There are a substantial number of clinical reports regarding the hypoglycemic action of berberine in Chinese literature reports<sup>[8]</sup>. A meta-analysis of berberine reported beneficial effects on blood glucose control in the treatment of T2DM patients similar to those obtained with conventional oral antidiabetic treatments<sup>[9]</sup>. One study confirms that administration of berberine (0.5 g three times/d) at the beginning of each major meal was able to reduce fasting blood glucose as well as postprandial blood glucose in adult patients with newly diagnosed T2DM. Glycated hemoglobin A1c (A1C) level was decreased by 2.0% with berberine treatment, which is comparable to that of metformin. In poorly controlled diabetic patients<sup>[8]</sup>, berberine regulates glucose metabolism possibly through multiple mechanisms and signal pathways such as increasing insulin sensitivity, activating the adenosine monophosphate- (AMP-) activated protein kinase (AMPK) pathway, modulating gut microbiota, inhibiting liver gluconeogenesis, stimulating glycolysis in peripheral tissue cells, promoting intestinal glucagon-like protein-1 secretion, upregulating hepatic low-density lipoprotein receptor mRNA expression, and increasing glucose transporter<sup>[10]</sup>.

The effects of berberine on lipid metabolism have been evaluated in animals and humans. A systematic review and meta-analysis of randomized controlled trials with berberine show that its administration produced a significant reduction in total cholesterol (mean difference -0.61 mmol/L; 95%CI: -0.83 to -0.39), triglycerides (mean difference -0.50 mmol/L; 95%CI: -0.69 to -0.31), and low-density lipoprotein cholesterol (LDL-C) (mean difference -0.65 mmol/L; 95%CI: -0.76 to -0.54) levels, with a remarkable increase in high-density lipoprotein cholesterol (HDL-C) (mean difference 0.05 mmol/L; 95%CI: 0.02 to 0.09)<sup>[11]</sup>. The lipid-lowering effect of berberine appears to be mainly due to stabilization of hepatic LDL receptor (LDL-R) in an extracellular signal-regulated kinase (ERK)-dependent manner and also by increasing transcriptional activity of LDL-R promoter by c-Jun N-terminal kinase (JNK) pathway. Berberine also activates AMPK while blocking the AMPK/ERK pathway, resulting in inhibition of lipid synthesis<sup>[7]</sup>.

Few reports in the literature affirm that berberine is able to decrease blood pressure in humans; however, vasorelaxant effects of berberine have been observed in different rat models<sup>[7]</sup>. Vasodilator effect of berberine is the result of its action on both endothelium and vascular smooth muscle. Other mechanisms suggested to be involved in the vasorelaxant effect of berberine are angiotensin-converting enzyme (ACE) inhibitor effect and direct release of nitric oxide (NO)/cyclic guanosine monophosphate (cGMP) from rat aortic rings,  $\alpha_1$ -adrenoreceptor antagonistic action in rat and rabbit aorta, potentiation of acetylcholine, activation

of K<sup>+</sup> channels and inhibition of intracellular calcium release, and blocking of L-type calcium channels<sup>[7]</sup>. A recent study showed that berberine could delay the onset and attenuate the severity of hypertension as well as to ameliorate hypertension-induced renal damage in spontaneously hypertensive rats (SHR). Furthermore, berberine could inhibit the activities of the renin-angiotensin system and pre-inflammatory cytokines such as interleukin (IL)-6, IL-17 and IL-23, which are involved in the pathophysiology of hypertension<sup>[12]</sup>.

Several clinical studies have reported the effect of berberine on obesity indicators such as body weight reduction, waist circumference or body mass index (BMI). A study in 116 patients with T2DM and dyslipidemia showed that berberine (1.0 g daily) compared with placebo for 3 mo decreased body weight from 68.7 ± 11.3 to 66.4 ± 11.8 kg<sup>[13]</sup>. This effect could be due to an inhibition of adipogenesis that may contribute to the anti-obesity activity of berberine. Since then, it has been shown to suppress adipocyte differentiation and reduce lipid accumulation in (3T3-L1) adipocytes. In cells treated with berberine, expression of several lipogenic genes including peroxisome proliferator-activated receptor gamma (PPAR<sub>γ</sub>), enhancer-binding protein alpha (EBP<sub>α</sub>), sterol regulatory element-binding protein 1 (SREBP-1c), fatty acid synthase, acetyl coenzyme A carboxylase, acyl-CoA synthase, lipoprotein lipase, and cluster of differentiation 36 were all suppressed<sup>[8]</sup>.

The above-mentioned findings show that berberine has excellent potential for prevention and treatment of MetS. A randomized, double-blind, placebo-controlled clinical trial carried out by our research group in 24 patients with a diagnosis of MetS showed that, after berberine administration, patients had a remission of 36% ( $P = 0.037$ ) in the presence of MetS and a significant decrease in waist circumference in females (106 ± 4 cm vs 103 ± 3 cm,  $P < 0.05$ ), systolic blood pressure (123 ± 7 mmHg vs 115 ± 9 mmHg,  $P < 0.01$ ), and triglycerides (2.4 ± 0.7 mmol/L vs 1.4 ± 0.5 mmol/L,  $P < 0.01$ ) in female and male patients<sup>[14]</sup>.

There is no effective dose for berberine; however, the therapeutic dosage for most clinical situations is 0.2-1.5 g/d for the treatment of various diseases, especially for T2DM<sup>[7]</sup>.

Berberine has been shown to be safe in the majority of clinical trials. In a low percentage of patients, berberine has been reported to cause nausea, vomiting, constipation, hypertension, respiratory failure and paresthesias; however, clinical evidence of such adverse effects is not often reported in the literature<sup>[7]</sup>.

The diverse pharmacological properties exhibited by berberine indicate that the alkaloid has definite potential as a drug in a wide spectrum of clinical applications that include MetS.

common tropical vegetable that has also been used in traditional medicine. The plant grows in tropical areas of Asia, Amazon, East Africa, India and the Caribbean<sup>[15]</sup>. Approximately 228 different compounds with possible medicinal properties have been isolated from bitter melon fruit, seeds, leaves, pericaps and endosperm. Among these, the most actively studied constituents shown to improve glycemic control include charantin, vicine, momordicin, and polypeptide-p. Polypeptide-p closely resembles bovine insulin with the exception of one extra amino acid, methionine<sup>[16]</sup>.

Several mechanisms of action have been proposed for its effects on glucose, lipids and blood pressure. Studies have shown that bitter melon inhibits the absorption of glucose by inhibiting  $\alpha$ -glucosidase, reduces Na<sup>+</sup>/K<sup>+</sup>-dependent absorption of glucose by the intestinal mucosa and also suppresses disaccharidase activity in the intestine<sup>[15,17]</sup>. Bitter melon repairs damaged  $\beta$ -cells, stimulates insulin secretion, and enhances insulin sensitivity. Enhancement in insulin sensitivity may be due to multiple factors such as inhibition of protein tyrosine phosphatase 1B (PTP-1B) activity in skeletal muscle, increase in the number and translocation of glucose transporter type 4 (GLUT4) receptors, increase in the rate of phosphorylation of insulin receptor substrate and enhancement in the activity of AMPK. AMPK inhibits cholesterol synthesis in liver by activating 3-hydroxy-3-methylglutaryl-coenzyme reductase. It also stimulates the synthesis and release of thyroid hormones and adiponectin<sup>[17]</sup>. Other proposed mechanisms for actions include decreased hepatic gluconeogenesis and increased hepatic glycogen synthesis<sup>[18]</sup>. PPARs are nuclear receptors that control lipid and carbohydrate metabolism. These receptors are regarded as important targets for treating MetS. In animal models, bitter melon upregulated PPAR<sub>γ</sub>- and PPAR<sub>α</sub>-mediated pathways<sup>[18]</sup>.

The hypoglycemic, hypolipidemic and antihypertensive effects of bitter melon have been reported in animal models and clinical trials. Male db/db mice (an animal model of obesity, diabetes, and dyslipidemia) were given sterile tap water as a control or bitter melon daily at a dosage of 150 mg/kg body weight for 5 wk. A1C levels were higher in control mice compared with the bitter melon-treated mice. Additionally, bitter melon reduced PTP-1B activity in skeletal muscle cytosol<sup>[19]</sup>. Normal and streptozotocin-induced diabetic rats were fed either with basal diet or a diet containing 10% bitter melon powder. Specific activities of intestinal disaccharidases were significantly increased during diabetes. Bitter melon supplementation in the diet clearly indicated amelioration in the activities of maltase and lactase during diabetes<sup>[20]</sup>. The effect of bitter melon at 10% level in the diet was evaluated in streptozotocin-induced diabetic rats. Amelioration of approximately 30% in fasting blood glucose was observed<sup>[21]</sup>. The aqueous extract powder of the fruit of bitter melon at a dose of 20 mg/kg body weight was also found to reduce fasting blood glucose by 48% in diabetic rats<sup>[22]</sup>.

## BITTER MELON

Bitter melon, also known as *Momordica charantia*, is a

To date, most published human clinical trials on bitter melon have focused on blood glucose control. A randomized, double-blind, active-control trial was conducted to assess the efficacy and safety of three doses of bitter melon compared with metformin. Patients were randomized into four groups to receive bitter melon 500 mg/d, 1000 mg/d, and 2000 mg/d or metformin 1000 mg/d. All patients were followed for 4 wk. There was a significant decline in fructosamine in the metformin group ( $16.8 \pm 40.6 \mu\text{mol/L}$ ) and the bitter melon 2000 mg/d group ( $-10.2 \pm 23.3 \mu\text{mol/L}$ )<sup>[23]</sup>. After adding bitter melon (800–1600 mg/d) to the current regimens (sulfonylureas and/or metformin) of 42 diabetic patients, fasting plasma glucose was reduced by  $26.9 \pm 40.8 \text{ mg/dL}$  ( $P < 0.001$ )<sup>[24]</sup>.

The effect of bitter melon on blood pressure and lipids has been evaluated in several experimental studies and only one clinical trial has aimed to investigate its effects on MetS. Acute intravenous administration of bitter melon aqueous extract produced dose-dependent, significant reductions in systemic arterial blood pressure and heart rates of normal and hypertensive Dahl salt-sensitive rats<sup>[25]</sup>. In another study, normal Sprague Dawley rats were divided into control and three test groups. Rats were administered one of three bitter melon preparations in food for 52 d: Chinese or Indian commercial preparations or an extract of bitter melon. All test groups lowered systolic, but not diastolic, blood pressure. Only the group with the extract significantly lowered ACE activity<sup>[26]</sup>. The methanol extract of bitter melon fruit was administered to diabetic rats for 30 d. A significant decrease in triglyceride and LDL-C and a significant increase in HDL-C were observed<sup>[27]</sup>. Bitter melon lowered plasma apolipoprotein B-100 and apolipoprotein B-48 levels in mice fed a high-fat diet and inhibited lipogenesis by downregulating lipogenic gene expression in adipose tissue of diet-induced obese mice<sup>[17]</sup>.

A preliminary open-label, single arm, uncontrolled supplementation trial was carried out in 42 participants to evaluate the effect of bitter melon supplementation (4.8 g/d for 3 mo) on MetS. Decrease in the incidence of MetS rate at the end of the supplementation period was significantly different from that at baseline (19.0%,  $P = 0.021$ ). The difference remained significant for 1 mo after cessation of supplementation ( $P = 0.047$ ). Except for waist circumference ( $-2.09 \text{ cm}$ ,  $P < 0.05$ ), the remaining four risk factors of MetS did not show significant decreases after bitter melon supplementation<sup>[18]</sup>.

An effective dose for bitter melon has not been established. In animal models the dose range has oscillated from 20 to 150 mg/kg body weight, whereas in clinical trials the dose has varied from 500 mg to 4800 mg per day<sup>[18,19,22,24]</sup>.

Few side effects have been associated with the use of bitter melon. The most commonly observed adverse effects include mild diarrhea and abdominal pain, which subside after discontinuation. Bitter melon use

is also contraindicated during pregnancy because of its abortifacient properties<sup>[16]</sup>.

Although the effect of bitter melon on glucose, blood pressure and lipids has been evaluated in several studies with significant results, only one clinical trial has assessed its effect on waist circumference as a primary outcome. Therefore, its effects on body weight remain to be studied in future clinical trials. The multiple mechanisms behind the hypoglycemic, hypolipidemic and antihypertensive effects of bitter melon and the results reported in previous studies provide a firm base for further well-designed randomized controlled trials to evaluate the efficacy of bitter melon on MetS.

## G. SYLVESTRE

*G. sylvestre* is a medicinal plant belonging to the Asclepiadaceae family popularly known as “gurmar” in Hindi, which means “sugar destroying”. It is a woody climber that grows in tropical forests in India and South East Asia. Its leaves exhibit a broad range of therapeutic effects due to its active ingredients referred to as gymnemic acids. These are a mixture of at least 17 different saponins, acidic glycosides and anthroquinones<sup>[28]</sup>. In Indian medicine it is used for its main antidiabetic effects; however, other important metabolic effects have emerged from various studies with potential for treating MetS<sup>[29,30]</sup>.

*G. sylvestre* helps to promote weight loss possibly through its ability to reduce cravings for sweets and also controls blood sugar levels. Chewing the leaves, rinsing the mouth with aqueous extracts, or topical application to the tongue selectively and reversibly inhibit the sensation of sweetness. Some investigations have suggested that gymnemic acid binds to the receptor located on the taste buds of the tongue and prevents activation by sugar molecules as well as suppressing sugar uptake, presumably by blocking sucrose receptors by one of its molecules, the gurmardin peptide<sup>[31,32]</sup>.

*G. sylvestre* has also been found to be useful against obesity in accordance with recent preclinical studies in a murine model of obesity where the anti-obesity effect of ethanolic or water-soluble fraction of *G. sylvestre* extract (120 mg/kg, orally for 21 d) was demonstrated in a high-fat diet (HFD)-induced murine model of obesity<sup>[33]</sup>. Another study with a standardized ethanolic *G. sylvestre* extract (200 mg/kg) administered for 28 d resulted in a significant reduction of BMI, organ weight and visceral fat pad weight, among other metabolic parameters<sup>[34]</sup>. *G. sylvestre* has also shown a decrease in body weight without rebound on Otsuka Long-Evans Tokushima Fatty rats<sup>[35]</sup>. Decreasing body weight in humans has been demonstrated in studies using *G. sylvestre* only in combination with various dietary supplements. Therefore, the resulting weight loss cannot be attributed to only *G. sylvestre*<sup>[36,37]</sup>.

Researchers have recently established that *G. sylvestre* does not block only sweet receptors on the taste buds of the mouth. It has the same inhibitory



activity on sodium-dependent glucose transporter 1 found in high levels in brush-border membranes of intestinal epithelial cells<sup>[38]</sup>.

The ability of *G. sylvestre* to lower blood glucose concentrations has been tested as a hypoglycemic agent in combination with insulin in humans, with encouraging results. A preliminary study shows that administration of 200 mg/d of *G. sylvestre* extract decreased the required insulin dose by 50% and lowered A1C in both type 1 and T2DM. It also increased the number of beta cells in the pancreas and therefore the internal production of insulin. When 400 mg/d of this extract is taken with conventional hypoglycemic drugs such as glyburide or tolbutamide, some patients were able to reduce the dose of the drug or even discontinue its use<sup>[39,40]</sup>. *In vivo* studies with oral administration of an extract of *G. sylvestre*, Om Santal Adivasi (OSA<sup>®</sup>) (1 g/d for 60 d) induced a significant increase in circulating insulin and C-peptide, which were associated with significant reductions in fasting and postprandial blood glucose. *In vitro* measurements using isolated human islets of Langerhans demonstrated direct stimulatory effects of OSA<sup>®</sup> on insulin secretion in human cells, consistent with an *in vivo* mode of action through enhancing insulin secretion. As a result, it also stabilizes blood sugar and decreases insulin doses. In fact, one patient with a disease duration of 10 years and another patient with a duration of 2 years and who were both using a total of 20 U of insulin a day were able to completely discontinue insulin at this point in the study<sup>[41]</sup>.

Individual chemical components of extract of *G. sylvestre* have also been shown to be potent and selective antagonists *in vitro* and *in vivo* for the  $\beta$  isoform of liver X receptor<sup>[42]</sup> in rats in whom *G. sylvestre* was administered at a dose of 200 mg/kg. Significant reductions in lipid levels and an increase in HDL-C have been reported<sup>[43]</sup>.

Compounds from the leaves of *G. sylvestre* may act as an endothelial synthase (eNOS) agonist. To further confirm the results, animal studies were performed with *G. sylvestre* leaves to demonstrate its future usefulness, not only in controlling blood glucose levels in diabetic patients but also to help avoid diabetic complications such as vascular diseases that occur due to decreased availability of NO<sup>[44]</sup>. One of the most active constituents of *G. sylvestre* is deacyl gymnemic acid (DAGA), which is associated with decreases in homeostasis model assessment (HOMA) insulin resistance, a surrogate marker of insulin resistance, suggesting treatment with DAGA at a dose of 200 mg/kg has beneficial effects on improvement in insulin sensitivity<sup>[30]</sup>. Conversely, in another study, systolic blood pressure was increased in SHR fed a high sucrose diet, but the clinical importance of this finding is unknown<sup>[37]</sup>.

Clinical studies investigating antidiabetic effects have typically used 200-1000 mg extract daily, standardized to contain 25% gymnemic acids<sup>[30,39,41]</sup>.

Adverse effects have not been reported in long-term studies in patients with type 1 diabetes<sup>[45]</sup>. However,

at high doses, hypoglycemia, weakness, excessive sweating and muscular dystrophy may occur<sup>[46]</sup>. On the other hand, due to its lipophilic character, *G. sylvestre* may inhibit intestinal absorption of oleic acid<sup>[47]</sup>. However, the United Nations Organizations has reported only one case of toxic hepatitis due to the use of *G. sylvestre*. Additional studies are needed to support its toxic effect<sup>[48]</sup>. The above-mentioned evidence supports the possibility of treating MetS with *G. sylvestre*, although more studies are needed.

## I. GABONENSIS

*I. gabonensis* belongs to the family Irvingiaceae. The tree of Irvingia, commonly known as mango bush, wild bush, dikanut or African mango, is native to Central and Occidental Africa<sup>[49]</sup>. Both the fruit and seeds of *I. gabonensis* are widely consumed in Africa as part of its gastronomy. It has recently been reported that roots, leaves and an extract of the seeds have medicinal properties.

*I. gabonensis* has been used for the treatment of diarrhea and to shorten the time of lactation in women. It is also administered for the treatment of colicky pain and dysentery. The tree bark has antibiotic properties and helps to heal dermal wounds produced by burning. It has also been administered for the treatment of toothache.

The use of an extract of *I. gabonensis* seeds has been studied as a source of dietary fiber useful to decrease glucose and cholesterol concentrations in diseases such as diabetes mellitus. Gastric emptying is delayed and absorption of glucose at the intestinal level is reduced, leading to better insulin sensitivity in tissues. This extract has also demonstrated to modify distribution of phospholipids, which lowers the plasma concentrations of total cholesterol and triglycerides<sup>[50]</sup>. Although the use of the extract of *I. gabonensis* has increased, no pharmacokinetic data have been reported.

Different studies have been carried out to determine the composition, antioxidant capacity, mechanism of action and effects of *I. gabonensis*. One study that aimed to identify the principal components of an extract of *I. gabonensis* seeds through high-resolution liquid chromatography coupled to mass spectrophotometry demonstrated that its principal components are ellagic acid, mono-, di-, and tri-O-methyl-ellagic and some long-chain glucosides<sup>[51]</sup>.

In relation to its antioxidant activity, a study was carried out to evaluate the antioxidant capacity of 14 species from Cameroon including *I. gabonensis*. Using different methanol extracts and two different assays to determine antioxidant capacity - the Folin assay and the ferric reduction potential assay - it was found that *I. gabonensis* has an elevated antioxidant concentration of approximately 202 mmol/100 g<sup>[52]</sup>.

Another experimental study was carried out with the aim of investigating the effect of an extract of *I. gabonensis* on inhibition of intracellular triglycerides

and the activity of the enzyme glycerol-3-phosphate in adipocytes 3T3-L1 of a murine model. Expression of some proteins typical of adipogenesis, leptin and adiponectin was also studied. Adipocytes were cultivated for 8 d after initiation of their differentiation and were treated with 0-250  $\mu\text{mol/L}$  of *I. gabonensis* for 12 and 24 h at 37 °C in an incubator with humidity at 5%. The results showed that *I. gabonensis* significantly inhibits adipogenesis in adipocytes. This effect appears to be mediated through a decrease in the expression of the PPAR $\gamma$  ( $P < 0.05$ ) and leptin ( $P < 0.05$ ). An increase in adiponectin expression was also found ( $P < 0.05$ )<sup>[53]</sup>.

An experimental study carried out in diabetic rats fed for 4 wk with a typical rat diet supplemented with *I. gabonensis* or cellulose found that both types of diets significantly reduced glucose, cholesterol and triglycerides concentrations and also increased HDL-C ( $P < 0.05$ )<sup>[54]</sup>.

These results agree with results reported in another experimental study where the potential of a seed extract of *I. gabonensis* was studied to decrease hyperglycemia and hyperlipidemia in a group of diabetic rats administered a diet supplemented with *I. gabonensis* for 4 wk. The results showed a significant decrease in glucose concentrations, food intake, total cholesterol, triglycerides and LDL-C levels. A significant increase in HDL-C was also reported ( $P < 0.05$ )<sup>[49]</sup>.

A study in which the effect of the administration of a viscous presentation of *I. gabonensis* seeds in diabetic rats was evaluated for 3 wk at a dose of 2 g/kg every 12 h showed that the extract decreased glucose concentrations ( $P < 0.05$ ), decreased activity of the enzymes pyruvate kinase and lactate dehydrogenase ( $P < 0.05$ ) and increased the activity of the enzyme glucose-6-phosphatase ( $P < 0.05$ ) compared with the control group<sup>[55]</sup>.

Another experimental study was carried out to evaluate the long-term effect of an aqueous extract of the bark of *I. gabonensis* administered daily to rabbits for 24 wk. At the end of the study, glucose concentration and body weight significantly decreased ( $P < 0.05$ )<sup>[56]</sup>.

Some clinical trials have been conducted to determine if *I. gabonensis* has an effect on body weight, glucose and lipid concentrations. A double-blind clinical trial carried out in 40 obese subjects who received *I. gabonensis* or placebo at a dose of 1.05 g three times/d for 1 mo showed that the administration of the extract of *I. gabonensis* decreased on average 5.25 kg of body weight ( $P < 0.001$ ). The subjects also showed a significant decrease of total cholesterol, LDL-C and triglycerides concentrations and increased their HDL-C<sup>[57]</sup>.

Another clinical trial was conducted in 102 overweight or obese subjects who were randomized into two groups: One group who received 150 mg of *I. gabonensis* 30 min prior to breakfast and dinner and the other received placebo at the same dose for 10 wk. The results showed a significant diminution on body weight, fat mass and waist circumference.

Significant differences were also found in plasma concentrations of total cholesterol, LDL-C, glucose, C-reactive protein, leptin and a significant increase was shown for adiponectin and HDL-C concentrations in the *I. gabonensis* group vs placebo<sup>[58]</sup>.

An approved dose has not yet been established for its use. A systematic review of three randomized controlled trials that evaluated the efficacy of *I. gabonensis* supplementation in the management of overweight and obesity found that the daily dosages differed from approximately 200 mg to approximately 3150 mg<sup>[59]</sup>.

Adverse events reported in some clinical trials regarding the use of *I. gabonensis* are headache, dry mouth, diarrhea, sleep disturbances, and constipation<sup>[60]</sup>. Acute toxicity studies have not reported any deaths after the 7-d administration of *I. gabonensis* at a dose of 1600 mg/kg in rats<sup>[59]</sup>.

The different studies performed either in animal models or as clinical trials suggest that the administration of *I. gabonensis* may be a promising option for the prevention and treatment of MetS.

## RESVERATROL

As a chemical compound, resveratrol (3,5,4-trihydroxystilbene) has been described since the 1940s when it was isolated for the first time from the roots of a white hellebore. Years later, it was extracted from the dried roots of a plant called *Polygonum cuspidatum*, which is often used in traditional Chinese medicine<sup>[61]</sup>.

Today it is known that resveratrol can be found in different quantities in > 70 plants and is also present in some foods and beverages such as nuts, berries, grapes, peanuts and their derivatives such as red wine. The quantity of resveratrol depends of different factors such crop type, geographical origin, and climate<sup>[62]</sup>.

In plants, resveratrol acts as a phytoalexin, a toxic compound produced by plants as a defense mechanism in response to the presence of pests and other stressful situations such as climate.

Resveratrol can be found in two different isomeric forms: *cis* and *trans*, the *cis* form being the more common used form due to its pharmacological properties<sup>[63]</sup>.

Despite the multiple therapeutic effects attributed to resveratrol, its pharmacokinetic characteristics are not favorable because of its poor bioavailability. It is rapidly metabolized and excreted<sup>[64]</sup>.

There is no evidence of the existence of specific receptors for resveratrol. However, resveratrol seems to accumulate in different tissues, mainly related with its absorption and metabolism such as duodenum, colon, liver and kidney<sup>[65-67]</sup>.

Although most of the studies carried out with resveratrol are in regard to its cardioprotective effect, there is evidence that resveratrol has other pharmacological properties in a wide range of chronic diseases such as cancer, T2DM, and degenerative diseases such as Alzheimer's as well as having antithrombotic,

antiosteoporotic and antimicrobial effects<sup>[63,67]</sup>. Resveratrol acts through different mechanisms. Similar to other polyphenols, resveratrol has an important antioxidant activity and interacts with different receptors, kinases and enzymes<sup>[68]</sup>. Some studies carried out in *in vivo* models reveal that resveratrol activates sirtuin 1 (SIRT1) and AMPK, both molecules implicated in metabolism regulation; therefore, resveratrol could be a new alternative for the prevention and treatment of MetS<sup>[69]</sup>.

Activation of SIRT1 by resveratrol decreases the activity of PPAR $\gamma$  and therefore adipogenesis, which decreases the number of adipocytes and thus obesity. Resveratrol also increases phosphorylation of the co-activator type 1 $\alpha$  of PPAR (PGC-1 $\alpha$ ) and cyclic adenine monophosphate (cAMP), which increases lipolysis. Resveratrol also enhances the activity of AMPK, which decreases the activity of acetyl CoA carboxylase by its phosphorylation, resulting in a decrease of lipogenesis that contributes to the control of obesity and dyslipidemia. Increase of the activity of AMPK stimulates phosphorylation of the myocyte enhancer factor 2 (MEF2), which results in a higher expression of GLUT4 and therefore a lower resistance to insulin and a diminution of glucose.

Finally, resveratrol increases the activity of endothelial eNOS and therefore the NO concentrations, which contributes to the vasodilation and indirectly to decreased blood pressure<sup>[70]</sup>. All these effects have been confirmed in different studies, both in animal models and in clinical trials.

A clinical trial was conducted in 11 males with obesity but without any other metabolic alteration. Patients received resveratrol or homologated placebo at a dose of 150 mg/d for 30 d. Results show that resveratrol activated AMPK at the muscular level and increases levels of SIRT1 and PGC-1 $\alpha$ , resulting in higher lipolysis of adipose tissue. A decrease in glucose, insulin and HOMA index was also demonstrated<sup>[71]</sup>. A meta-analysis carried out with 11 clinical trials found that resveratrol administrated at different doses for at least 2 wk in patients with diabetes decreases fasting glucose, insulin, A1C and insulin resistance evaluated through HOMA index, but this meta-analysis did not find any differences in patients without diabetes<sup>[72]</sup>.

Although the information obtained about the effects of resveratrol on cholesterol and triglycerides concentrations is inconclusive, some studies performed in animal models with MetS have shown that resveratrol at different doses reduces atherosclerotic plaque formation, total cholesterol and triglycerides<sup>[73,61]</sup>. Clinical trials reported in obese patients have not found any significant differences in lipid profile after resveratrol administration<sup>[71,74]</sup>.

Our research group<sup>[75]</sup> conducted a randomized, double-blind, placebo-controlled clinical trial in 24 patients with a diagnosis of MetS in accordance with the International Diabetes Federation modified criteria. Resveratrol or homologated placebo was administrated for 90 d at a dose of 500 mg three times per day. After

resveratrol administration, significant differences were found in total weight ( $94.4 \pm 13.2$  kg vs  $90.5 \pm 12.3$  kg,  $P = 0.007$ ), BMI ( $35.6 \pm 3.2$  kg/m<sup>2</sup> vs  $34.3 \pm 3.0$  kg/m<sup>2</sup>,  $P = 0.006$ ), fat mass ( $41.2 \pm 7.9$  kg vs  $38.8 \pm 6.0$  kg,  $P = 0.001$ ), and waist circumference ( $109 \pm 9$  cm vs  $105 \pm 10$  cm,  $P = 0.004$ ). There were also significant differences in area under the curve (AUC) of insulin ( $48418 \pm 22707$  pmol/L vs  $26473 \pm 8273$  pmol/L,  $P = 0.003$ ) and total insulin secretion evaluated through insulinogenic index ( $0.48 \pm 0.22$  pmol/L vs  $0.28 \pm 0.08$  pmol/L,  $P = 0.004$ ).

An approved dose has not yet been established for its use. In a meta-analysis where the effect of resveratrol on glucose control and insulin sensitivity was evaluated, a dose range from 8 to 1500 mg/dL was found<sup>[72]</sup>.

Some adverse effects reported due to the use of resveratrol are headache, abdominal pain and general malaise<sup>[75]</sup>. At high doses (2000 mg twice daily for 1 wk), a clinical trial reported statistically, but not clinically significant, increased serum bilirubin and potassium concentrations<sup>[76]</sup>. Daily dosing of 100 mg for 4 wk did not change these values<sup>[77]</sup>.

These results lead to the conclusion that resveratrol could be an option for the treatment of MetS due to the decrease of obesity and by controlling the hypersecretion of insulin characteristic of this group of patients.

## URSOLIC ACID

Ursolic acid is a pentacyclic triterpene carboxylic acid present as a free acid or as an aglycone part of saponins<sup>[78]</sup> and can be obtained naturally or synthetically<sup>[79]</sup>. It is also known as urson, prunol, micromerol or malol<sup>[80]</sup>. This compound was considered inactive; however, in recent years interest has been sparked due to the multiple and varied effects of ursolic acid<sup>[79,81]</sup>. Evidence for this substance appears promising for the treatment of MetS.

The main sources of ursolic acid include components of certain fruits, herbs and plants. Ursolic acid is found in apple peel, cranberry juice and grape skin. It is also found in some common spices like rosemary, thyme and oregano and has been identified in Ayurvedic herbs such as Holy Basil, some traditional Chinese medicinal herbs including Jujuba zizyphus, and in yerba mate and sage. Ursolic acid also is found in some herbs that have attributed antidiabetic effects and is found in small amounts in the leaves of some plants<sup>[82,83]</sup>.

Ursolic acid is formed by 30 carbons distributed in five rings of six carbons and has an hydroxyl group at carbon 3, a carboxyl group at carbon 28 and a double bond at carbon 12 and 13. Its chemical formula is C<sub>30</sub>H<sub>48</sub>O<sub>3</sub><sup>[84]</sup>. Some structurally related compounds of ursolic acid include its isomer, oleanolic acid, in addition to corosolic, maslinic, latanolic, pomolic, camarinic and pomolic acids<sup>[85]</sup>. These compounds share common characteristics of pentacyclic triterpenoids with

apparently similar effects, although differing from each other in strength<sup>[85]</sup>.

Physicochemical properties of ursolic acid give it great stability. Ursolic acid has a molecular weight of 456.70032 g/mol. Its melting point is 269–271 °C. It has an optical activity of +34° at a concentration of 0.20 g/100 mL in methanol and a molar solubility in pure water at pH 7 and 25 °C of  $1.11 \times 10^{-5}$  mg/L<sup>[80,84]</sup>.

Evidence demonstrates positive effects *in vitro* and *in vivo* through various mechanisms in glucose and lipid metabolism as well as in body weight and visceral fat usually altered in MetS.

Ursolic acid inhibits the enzyme PTP1B, promoting phosphorylation of the insulin receptor *in vitro*, thereby stimulating glucose uptake<sup>[86,87]</sup>. PTPB1 is an enzyme associated with the endoplasmic reticulum and plays a key role in signaling metabolic pathways that interacts and dephosphorylates insulin receptor and leptin, causing downregulation signaling of both receptors in modulating the mitogenic actions of insulin<sup>[88]</sup>.

Translocation of GLUT4 is increased by ursolic acid as part of the action on the insulin receptor and manages to improve glucose uptake. GLUT4 is the principal glucose transporter protein and thus plays a key role in regulating whole body glucose homeostasis<sup>[88]</sup>.

Ursolic acid appears to inhibit the  $\alpha$ -amylase enzyme, an enzyme that hydrolyzes  $\alpha$ -links of large polysaccharides such as starch and glycogen to yield glucose and maltose. Inhibition of  $\alpha$ -amylase has been shown to lower blood glucose levels due to lowering the breakdown and absorption of starch<sup>[89]</sup>.

Ursolic acid reduces the activity of aldose reductase and sorbitol dehydrogenase<sup>[90,91]</sup>. These enzymes catalyze the reduction of hexoses. In the presence of hyperglycemia, aldose reductase converts glucose to sorbitol. The latter is metabolized to fructose by sorbitol dehydrogenase. During this process, the production of sorbitol and fructose occurs. Reduced nicotinamide adenine dinucleotide phosphate is decreased and nicotinamide adenine dinucleotide phosphate is increased<sup>[91]</sup>. Sorbitol increases intracellular osmotic pressure and damages tissues by cell edema; fructose causes protein fructosylation<sup>[90]</sup>.

The increase in the glyoxalase system produced by ursolic acid represents the decrease of cytotoxicity and chronic complications caused by methylglyoxal, a toxic metabolite produced as a by-product of metabolism. This detoxification reaction is carried out by the glyoxalase system<sup>[92]</sup>.

Administration of ursolic acid was associated with decreased adipocyte differentiation<sup>[93]</sup>. Adipocytes synthesize and release a wide variety of peptide and non-peptide substances and also store and mobilize triglycerides, cholesterol and retinoids. Lipid-laden adipocytes can be emptied and extended, forming cells that resemble their predecessors not only in appearance but also for its potential for multiplication. This change reflects fully differentiated adipocyte regression to an earlier or less mature, but complete, stage<sup>[93]</sup>.

Overregulation of the c-Cbl associated protein (CAP) was observed in adipocytes treated with ursolic acid<sup>[94]</sup>. CAP is expressed only in insulin-sensitive tissues (adipose, liver and muscle). Increase in transcription of CAP is directly related to greater sensitivity to insulin in adipocytes. It is postulated that CAP would facilitate phosphorylation of c-Cbl by the insulin receptor, allowing the union of c-Cbl to the insulin-dependent tyrosine kinase. The relationship of CAP is an example of a direct molecular link between PPAR $\gamma$  sensitivity and insulin in adipose tissue<sup>[94]</sup>.

Through the activation of protein kinase A, ursolic acid appears to increase lipolysis *in vitro* as well as to decrease hormone-sensitive lipase and perilipin activity<sup>[93]</sup>. Lipolysis favors the production of energy from fatty acids into the mitochondria, enabling the generation of free fatty acids from triglycerides stored in adipocytes of white adipose tissue. As a result, there is an activation of fatty acids as well as a translocation to the mitochondria from tissues such as muscle and brown adipose tissue. As a final result, the production of energy occurs from  $\beta$ -oxidation of fatty acids in mitochondria and in some cases in the peroxisome<sup>[93]</sup>.

There is no established dose for ursolic acid. Animal studies have found benefits with ursolic acid at 0.05%–0.2% of the diet<sup>[86–93]</sup>, which is about 10–40 mg/kg based on their weight and food intake. In clinical trials, a 150-mg dose one to three times a day has been used, providing a maximum of 450 mg and revealing some biological activity.

No adverse effects have been associated with ursolic acid in humans. However, studies in animals have reported that ursolic acid at very high doses resulted in a decrease of sperm motility, cell death and DNA damage<sup>[95]</sup>. Due to the beneficial effects of ursolic acid on several components of the MetS, its clinical administration should be further studied.

## CONCLUSION

Nutraceutical therapies such as berberine, bitter melon, *G. sylvestre*, *I. gabonensis*, resveratrol and ursolic acid have demonstrated substantial scientific information regarding their safety and beneficial effects to be comprehensively considered for treating patients with MetS. Berberine and resveratrol, which already have been studied in patients with MetS, have demonstrated valuable results. For the remainder of the nutraceuticals presented in this review, it may be necessary to perform more in-depth studies to be clinically recommended.

## REFERENCES

- 1 **Moreira GC**, Cipullo JP, Ciorlia LA, Cesarino CB, Vilela-Martin JF. Prevalence of metabolic syndrome: association with risk factors and cardiovascular complications in an urban population. *PLoS One* 2014; 9: e105056 [PMID: 25180496 DOI: 10.1371/journal.pone.0105056]
- 2 **Alberti KG**, Zimmet P, Shaw J. Metabolic syndrome—a new worldwide definition. A Consensus Statement from the International



- Diabetes Federation. *Diabet Med* 2006; **23**: 469-480 [PMID: 16681555 DOI: 10.1111/j.1464-5491.2006.01858.x]
- 3 **Tarantino G**, Finelli C. What about non-alcoholic fatty liver disease as a new criterion to define metabolic syndrome? *World J Gastroenterol* 2013; **19**: 3375-3384 [PMID: 23801829 DOI: 10.3748/wjg.v19.i22.3375]
  - 4 **González-Ortiz M**, Martínez-Abundis E, Jacques-Camarena O, Hernández-González SO, Valera-González IG, Ramos-Zavala MG. Prevalence of metabolic syndrome in adults with excess of adiposity: comparison of the Adult Treatment Panel III criteria with the International Diabetes Federation definition. *Acta Diabetol* 2006; **43**: 84-86 [PMID: 17143786 DOI: 10.1007/s00592-006-0218-2]
  - 5 **González-Ortiz M**, Martínez-Abundis E, Robles-Cervantes JA, Ramos-Zavala MG. Effect of xenatide on fat deposition and a metabolic profile in patients with metabolic syndrome. *Metab Syndr Relat Disord* 2011; **9**: 31-34 [PMID: 20874425 DOI: 10.1089/met.2010.0025]
  - 6 **Tillhon M**, Guamán Ortiz LM, Lombardi P, Scovassi AI. Berberine: new perspectives for old remedies. *Biochem Pharmacol* 2012; **84**: 1260-1267 [PMID: 22842630 DOI: 10.1016/j.bcp.2012.07.018]
  - 7 **Derosa G**, Maffioli P, Cicero AF. Berberine on metabolic and cardiovascular risk factors: an analysis from preclinical evidences to clinical trials. *Expert Opin Biol Ther* 2012; **12**: 1113-1124 [PMID: 22780092 DOI: 10.1517/14712598.2012.704014]
  - 8 **Yin J**, Zhang H, Ye J. Traditional chinese medicine in treatment of metabolic syndrome. *Endocr Metab Immune Disord Drug Targets* 2008; **8**: 99-111 [PMID: 18537696 DOI: 10.2174/187153008784534330]
  - 9 **Dong H**, Wang N, Zhao L, Lu F. Berberine in the treatment of type 2 diabetes mellitus: a systemic review and meta-analysis. *Evid Based Complement Alternat Med* 2012; **2012**: 591654 [PMID: 23118793 DOI: 10.1155/2012/591654]
  - 10 **Pang B**, Zhao LH, Zhou Q, Zhao TY, Wang H, Gu CJ, Tong XL. Application of berberine on treating type 2 diabetes mellitus. *Int J Endocrinol* 2015; **2015**: 905749 [PMID: 25861268 DOI: 10.1155/2015/905749]
  - 11 **Dong H**, Zhao Y, Zhao L, Lu F. The effects of berberine on blood lipids: a systemic review and meta-analysis of randomized controlled trials. *Planta Med* 2013; **79**: 437-446 [PMID: 23512497 DOI: 10.1055/s-0032-1328321]
  - 12 **Guo Z**, Sun H, Zhang H, Zhang Y. Anti-hypertensive and renoprotective effects of berberine in spontaneously hypertensive rats. *Clin Exp Hypertens* 2015; **37**: 332-339 [PMID: 25867076 DOI: 10.3109/10641963.2014.972560]
  - 13 **Zhang Y**, Li X, Zou D, Liu W, Yang J, Zhu N, Huo L, Wang M, Hong J, Wu P, Ren G, Ning G. Treatment of type 2 diabetes and dyslipidemia with the natural plant alkaloid berberine. *J Clin Endocrinol Metab* 2008; **93**: 2559-2565 [PMID: 18397984 DOI: 10.1210/jc.2007-2404]
  - 14 **Pérez-Rubio KG**, González-Ortiz M, Martínez-Abundis E, Robles-Cervantes JA, Espinel-Bermúdez MC. Effect of berberine administration on metabolic syndrome, insulin sensitivity, and insulin secretion. *Metab Syndr Relat Disord* 2013; **11**: 366-369 [PMID: 23808999 DOI: 10.1089/met.2012.0183]
  - 15 **Grover JK**, Yadav SP. Pharmacological actions and potential uses of Momordica charantia: a review. *J Ethnopharmacol* 2004; **93**: 123-132 [PMID: 15182917 DOI: 10.1016/j.jep.2004.03.035]
  - 16 **Efird JT**, Choi YM, Davies SW, Mehra S, Anderson EJ, Katunga LA. Potential for improved glycemic control with dietary Momordica charantia in patients with insulin resistance and pre-diabetes. *Int J Environ Res Public Health* 2014; **11**: 2328-2345 [PMID: 24566057 DOI: 10.3390/ijerph110202328]
  - 17 **Chaturvedi P**. Antidiabetic potentials of Momordica charantia: multiple mechanisms behind the effects. *J Med Food* 2012; **15**: 101-107 [PMID: 22191631 DOI: 10.1089/jmf.2010.0258]
  - 18 **Tsai CH**, Chen EC, Tsay HS, Huang CJ. Wild bitter gourd improves metabolic syndrome: a preliminary dietary supplementation trial. *Nutr J* 2012; **11**: 4 [PMID: 22243626 DOI: 10.1186/1475-2891-11-4]
  - 19 **Klomann SD**, Mueller AS, Pallauf J, Krawinkel MB. Antidiabetic effects of bitter gourd extracts in insulin-resistant db/db mice. *Br J Nutr* 2010; **104**: 1613-1620 [PMID: 20615270 DOI: 10.1017/S0007114510002680]
  - 20 **Kumar Shetty A**, Suresh Kumar G, Veerayya Salimath P. Bitter gourd (Momordica charantia) modulates activities of intestinal and renal disaccharidases in streptozotocin-induced diabetic rats. *Mol Nutr Food Res* 2005; **49**: 791-796 [PMID: 16007724 DOI: 10.1002/mnfr.200500035]
  - 21 **Shetty AK**, Kumar GS, Sambaiah K, Salimath PV. Effect of bitter gourd (Momordica charantia) on glycaemic status in streptozotocin induced diabetic rats. *Plant Foods Hum Nutr* 2005; **60**: 109-112 [PMID: 16187012 DOI: 10.1007/s11130-005-6837-x]
  - 22 **Virdi J**, Sivakami S, Shahani S, Suthar AC, Banavalikar MM, Biyani MK. Antihyperglycemic effects of three extracts from Momordica charantia. *J Ethnopharmacol* 2003; **88**: 107-111 [PMID: 12902059 DOI: 10.1016/S0378-8741(03)00184-3]
  - 23 **Fuangchan A**, Sonthisombat P, Seubnukarn T, Chanouan R, Chotchaisuwat P, Sirigulsatien V, Ingkaninan K, Plianbangchang P, Haines ST. Hypoglycemic effect of bitter melon compared with metformin in newly diagnosed type 2 diabetes patients. *J Ethnopharmacol* 2011; **134**: 422-428 [PMID: 21211558 DOI: 10.1016/j.jep.2010.12.045]
  - 24 **Fuangchan A**, Seubnukarn T, Jungpattanawadee D, Sonthisombat P, Ingkaninan K, Plianbangchang P, Haines ST. Retrospective study on the use of bitter melon for type 2 diabetes at Dnsai Crown Prince Hospital, Thailand. *Srinagarind Med J* 2009; **24**: 332-338
  - 25 **Ojewole JA**, Adewole SO, Olayiwola G. Hypoglycaemic and hypotensive effects of Momordica charantia Linn (Cucurbitaceae) whole-plant aqueous extract in rats. *Cardiovasc J S Afr* 2006; **17**: 227-232 [PMID: 17117226]
  - 26 **Clouatre DL**, Rao SN, Preuss HG. Bitter melon extracts in diabetic and normal rats favorably influence blood glucose and blood pressure regulation. *J Med Food* 2011; **14**: 1496-1504 [PMID: 21861717 DOI: 10.1089/jmf.2010.0276]
  - 27 **Chaturvedi P**, George S, Milinganyo M, Tripathi YB. Effect of Momordica charantia on lipid profile and oral glucose tolerance in diabetic rats. *Phytother Res* 2004; **18**: 954-956 [PMID: 15597317 DOI: 10.1002/ptr.1589]
  - 28 **Sinsheimer JE**, Rao GS. Constituents from Gymnema sylvestre leaves. VI. Acylated genins of the gymnemic acids--isolated and preliminary characterization. *J Pharm Sci* 1970; **59**: 629-632 [PMID: 5450284 DOI: 10.1002/jps.2600590511]
  - 29 **Chakraborty D**, Ghosh S, Bishayee K, Mukherjee A, Sikdar S, Khuda-Bukhsh AR. Antihyperglycemic drug Gymnema sylvestre also shows anticancer potentials in human melanoma A375 cells via reactive oxygen species generation and mitochondria-dependent caspase pathway. *Integr Cancer Ther* 2013; **12**: 433-441 [PMID: 23615751 DOI: 10.1177/1534735413485419]
  - 30 **Bhansali S**, Shafiq N, Pandhi P, Singh AP, Singh PK, Sharma S, Malhotra S. Effect of a deacyl gymnemic acid on glucose homeostasis & metabolic parameters in a rat model of metabolic syndrome. *Indian J Med Res* 2013; **137**: 1174-1179 [PMID: 23852298 DOI: 10.1016/0378-8741(83)90021-1]
  - 31 **Schroeder JA**, Flannery-Schroeder E. Use of the Herb Gymnema sylvestre to Illustrate the Principles of Gustatory Sensation: An Undergraduate Neuroscience Laboratory Exercise. *J Undergrad Neurosci Educ* 2005; **3**: A59-A62 [PMID: 23493970]
  - 32 **Warren RP**, Warren RM, Weninger MG. Inhibition of the sweet taste by Gymnema sylvestre. *Nature* 1969; **223**: 94-95 [PMID: 5792442 DOI: 10.1038/223094a0]
  - 33 **Kumar V**, Bhandari U, Tripathi CD, Khanna G. Anti-obesity effect of Gymnema sylvestre extract on high fat diet-induced obesity in Wistar rats. *Drug Res (Stuttg)* 2013; **63**: 625-632 [PMID: 23842942 DOI: 10.1055/s-0033-1349852]
  - 34 **Kumar V**, Bhandari U, Tripathi CD, Khanna G. Evaluation of antiobesity and cardioprotective effect of Gymnema sylvestre extract in murine model. *Indian J Pharmacol* 2012; **44**: 607-613 [PMID: 23112423 DOI: 10.4103/0253-7613.100387]
  - 35 **Nielubowicz J**. Magic of the physician's word. *Pol Tyg Lek* 1991; **46**: 679-684 [PMID: 1669131 DOI: 10.1007/s11010-005-9049-7]

- 36 **Woodgate DE**, Conquer JA. Effects of a stimulant-free dietary supplement on body weight and fat loss in obese adults: a six-week exploratory study. *Curr Ther Res Clin Exp* 2003; **64**: 248-262 [PMID: 24944372 DOI: 10.1016/S0011-393X(03)00058-4]
- 37 **Preuss HG**, Jarrell ST, Scheckenbach R, Lieberman S, Anderson RA. Comparative effects of chromium, vanadium and gymnema sylvestre on sugar-induced blood pressure elevations in SHR. *J Am Coll Nutr* 1998; **17**: 116-123 [PMID: 9550454 DOI: 10.1080/07315724.1998.10718736]
- 38 **Wang Y**, Dawid C, Kottra G, Daniel H, Hofmann T. Gymnemic acids inhibit sodium-dependent glucose transporter 1. *J Agric Food Chem* 2014; **62**: 5925-5931 [PMID: 24856809 DOI: 10.1021/jf501766u]
- 39 **Baskaran K**, Kizar Ahmath B, Radha Shanmugasundaram K, Shanmugasundaram ER. Antidiabetic effect of a leaf extract from *Gymnema sylvestre* in non-insulin-dependent diabetes mellitus patients. *J Ethnopharmacol* 1990; **30**: 295-300 [PMID: 2259217 DOI: 10.1016/0378-8741(90)90108-6]
- 40 **Shanmugasundaram ER**, Rajeswari G, Baskaran K, Rajesh Kumar BR, Radha Shanmugasundaram K, Kizar Ahmath B. Use of *Gymnema sylvestre* leaf extract in the control of blood glucose in insulin-dependent diabetes mellitus. *J Ethnopharmacol* 1990; **30**: 281-294 [PMID: 2259216 DOI: 10.1016/0378-8741(90)90107-5]
- 41 **Al-Romaiyan A**, Liu B, Asare-Anane H, Maity CR, Chatterjee SK, Koley N, Biswas T, Chatterji AK, Huang GC, Amiel SA, Persaud SJ, Jones PM. A novel *Gymnema sylvestre* extract stimulates insulin secretion from human islets in vivo and in vitro. *Phytother Res* 2010; **24**: 1370-1376 [PMID: 20812281 DOI: 10.1002/ptr.3125]
- 42 **Renga B**, Festa C, De Marino S, Di Micco S, D'Auria MV, Bifulco G, Fiorucci S, Zampella A. Molecular decodification of gymnemic acids from *Gymnema sylvestre*. Discovery of a new class of liver X receptor antagonists. *Steroids* 2015; **96**: 121-131 [PMID: 25668616 DOI: 10.1016/j.steroids.2015.01.024]
- 43 **Rachh PR**, Rachh MR, Ghadiya NR, Modi DC, Modi KP, Patel NM, Rupareliya MT. Antihyperlipidemic Activity of *Gymnema sylvestre* R. Br. Leaf Extract on Rats Fed with High Cholesterol Diet. *Inter J Pharmacol* 2010; **6**: 138-141 [DOI: 10.3923/ijp.2010.138.141]
- 44 **Khan KA**, Shabnam D, Mohammed AS. Molecular docking and preclinical studies of *Gymnema sylvestre* on endothelial nitric oxide synthase (enos) in Type-2 diabetes related complications. *J Young Pharm* 2014; **6**: 25-32 [DOI: 10.5530/jyp.2014.4.5]
- 45 **Thakur GS**, Sharma R, Sanodiya BS, Pandey M, Prasad GBKS, Bisen PS. *Gymnema sylvestre*: An Alternative Therapeutic Agent for Management of Diabetes. *JAPS* 2012; **2**: 1-6 [DOI: 10.7324/JAPS.2012.21201]
- 46 **Tiwari P**, Mishra BN, Sangwan NS. Phytochemical and pharmacological properties of *Gymnema sylvestre*: an important medicinal plant. *Biomed Res Int* 2014; **2014**: 1-18 [DOI: 10.1155/2014/830285]
- 47 **El Shafey AAM**, El-Ezabi MM, Seleim MME, Ouda HHM, Ibrahim DS. Effect of *Gymnema sylvestre* R. Br. leaves extract on certain physiological parameters of diabetic rats. *J King Saud Univ* 2013; **25**: 135-141 [DOI: 10.1016/j.jksus.2012.11.001]
- 48 **Song C**, Yang Z, Zhong M, Chen Z. Sericin protects against diabetes-induced injuries in sciatic nerve and related nerve cells. *Neural Regen Res* 2013; **8**: 506-513 [PMID: 25206693 DOI: 10.3969/j.issn.1673-5374.2013.06.003]
- 49 **Dzeuffiet PD**, Ngeutse FD, Dimo T, Tédong L, Ngueguim TF, Tchamadeu MC, Nkouambou NC, Sokeng SD, Kamtchouing P. Hypoglycemic and hypolipidemic effects of *Irvingia gabonensis* in diabetic rats. *Pharmacologyonline* 2009; **2**: 957-962
- 50 **Ainge L**, Brown N. Bush Mango (*Irvingia gabonensis* and *I. wombolu*). In: Clark LE, Sunderland TC. The Key Non-Timber Forest Products of Central Africa: State of the Knowledge. USAID, Bureau for Africa, Office of Sustainable Development, 2004: 15-35
- 51 **Sun J**, Chen P. Ultra high-performance liquid chromatography with high-resolution mass spectrometry analysis of African mango (*Irvingia gabonensis*) seeds, extract, and related dietary supplements. *J Agric Food Chem* 2012; **60**: 8703-8709 [PMID: 22880691 DOI: 10.1021/jf301703u]
- 52 **Agbor GA**, Oben JE, Ngogang JY, Xinxing C, Vinson JA. Antioxidant capacity of some herbs/spices from cameroon: a comparative study of two methods. *J Agric Food Chem* 2005; **53**: 6819-6824 [PMID: 16104805 DOI: 10.1021/jf050445c]
- 53 **Oben JE**, Ngondi JL, Blum K. Inhibition of *Irvingia gabonensis* seed extract (OB131) on adipogenesis as mediated via down regulation of the PPARGamma and leptin genes and up-regulation of the adiponectin gene. *Lipids Health Dis* 2008; **7**: 44 [PMID: 19014517 DOI: 10.1186/1476-511X-7-44]
- 54 **Omoriyi F**, Adamson I. Effect of supplements of dikanut (*Irvingia gabonensis*) and cellulose on plasma lipids and composition of hepatic phospholipids in streptozotocin-induced diabetic rats. *Nutrition Research* 1994; **14**: 537-544 [DOI: 10.1016/S0271-5317(05)80217-9]
- 55 **Ozula RI**, Eriyamremu GE, Okene EO, Ochei U. Hypoglycaemic effects of viscous preparation of *Irvingia gabonensis* (Dikanut) seeds in Streptozotocin-Induced Diabetic Wistar Rats. *Journal of Herbs, Spices & Medical Plants* 2014; **12**: 1-9 [DOI: 10.1300/J044v12n04\_01]
- 56 **Omonkhua AA**, Onoagbe IO. Effects of long-term oral administration of aqueous extracts of *Irvingia gabonensis* bark on blood glucose and liver profile of normal rabbits. *Journal of Medicinal Plants Research* 2012; **6**: 2581-2589 [DOI: 10.5897/JMPR11.561]
- 57 **Ngondi JL**, Oben JE, Minka SR. The effect of *Irvingia gabonensis* seeds on body weight and blood lipids of obese subjects in Cameroon. *Lipids Health Dis* 2005; **4**: 12 [PMID: 15916709 DOI: 10.1186/1476-511X-4-12]
- 58 **Ngondi JL**, Etoundi BC, Nyangono CB, Mbofung CM, Oben JE. IGOB131, a novel seed extract of the West African plant *Irvingia gabonensis*, significantly reduces body weight and improves metabolic parameters in overweight humans in a randomized double-blind placebo controlled investigation. *Lipids Health Dis* 2009; **8**: 7 [PMID: 19254366 DOI: 10.1186/1476-511X-8-7]
- 59 **Onakpoya I**, Davies L, Posadzki P, Ernst E. The efficacy of *Irvingia gabonensis* supplementation in the management of overweight and obesity: a systematic review of randomized controlled trials. *J Diet Suppl* 2013; **10**: 29-38 [PMID: 23419021]
- 60 African Mango. Accessed 2014 December 15. Available from: URL: <http://www.drugs.com/npp/african-mango.html>
- 61 **Baur JA**, Sinclair DA. Therapeutic potential of resveratrol: the in vivo evidence. *Nat Rev Drug Discov* 2006; **5**: 493-506 [PMID: 16732220 DOI: 10.1038/nrd2060]
- 62 **Zhang J**. Resveratrol inhibits insulin responses in a SirT1-independent pathway. *Biochem J* 2006; **397**: 519-527 [PMID: 16626303 DOI: 10.1042/BJ20050977]
- 63 **Millán-Parrila F**, Serrano-San Miguel G. Resveratrol nuevos retos en el tratamiento anti-envejecimiento. *Salud Estética* 2008; **5**: 1-5
- 64 **Kaldas MI**, Walle UK, Walle T. Resveratrol transport and metabolism by human intestinal Caco-2 cells. *J Pharm Pharmacol* 2003; **55**: 307-312 [PMID: 12724035 DOI: 10.1211/002235702612]
- 65 **Walle T**. Bioavailability of resveratrol. *Ann N Y Acad Sci* 2011; **1215**: 9-15 [PMID: 21261636 DOI: 10.1111/j.1749-6632.2010.05842.x]
- 66 **Timmers S**, Auwerx J, Schrauwen P. The journey of resveratrol from yeast to human. *Aging (Albany NY)* 2012; **4**: 146-158 [PMID: 22436213]
- 67 **Northrop MW**, Piper GM. A study of diets of patients in a prenatal clinic with an attempt to correlate dietary adequacy with physical findings. *Northwest Med* 1947; **46**: 294-298 [PMID: 20291649]
- 68 **Beaucloux JL**, Nivet-Antoine V, Giral P. Resveratrol: a relevant pharmacological approach for the treatment of metabolic syndrome? *Curr Opin Clin Nutr Metab Care* 2010; **13**: 729-736 [PMID: 20823772 DOI: 10.1097/MCO.0b013e32833ef291]
- 69 **Smoliga JM**, Baur JA, Hausenblas HA. Resveratrol and health—a comprehensive review of human clinical trials. *Mol Nutr Food Res* 2011; **55**: 1129-1141 [PMID: 21688389 DOI: 10.1002/mnfr.201100143]
- 70 **Baile CA**, Yang JY, Rayalam S, Hartzell DL, Lai CY, Andersen C, Della-Fera MA. Effect of resveratrol on fat mobilization. *Ann N Y Acad Sci* 2011; **1215**: 40-47 [PMID: 21261640 DOI: 10.1111/j.1749-6632.2010.05845.x]

- 71 Timmers S, Konings E, Bilet L, Houtkooper RH, van de Weijer T, Goossens GH, Hoeks J, van der Krieken S, Ryu D, Kersten S, Moonen-Kornips E, Hesselink MK, Kunz I, Schrauwen-Hinderling VB, Blaak EE, Auwerx J, Schrauwen P. Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans. *Cell Metab* 2011; **14**: 612-622 [PMID: 22055504 DOI: 10.1016/j.cmet.2011.10.002]
- 72 Liu K, Zhou R, Wang B, Mi MT. Effect of resveratrol on glucose control and insulin sensitivity: a meta-analysis of 11 randomized controlled trials. *Am J Clin Nutr* 2014; **99**: 1510-1519 [PMID: 24695890 DOI: 10.3945/ajcn.113.082024]
- 73 Robich MP, Osipov RM, Chu LM, Han Y, Feng J, Nezafat R, Clements RT, Manning WJ, Sellke FW. Resveratrol modifies risk factors for coronary artery disease in swine with metabolic syndrome and myocardial ischemia. *Eur J Pharmacol* 2011; **664**: 45-53 [PMID: 21575630 DOI: 10.1016/j.ejphar.2011.04.059]
- 74 Poulsen MM, Vestergaard PF, Clasen BF, Radko Y, Christensen LP, Stødkilde-Jørgensen H, Møller N, Jessen N, Pedersen SB, Jørgensen JO. High-dose resveratrol supplementation in obese men: an investigator-initiated, randomized, placebo-controlled clinical trial of substrate metabolism, insulin sensitivity, and body composition. *Diabetes* 2013; **62**: 1186-1195 [PMID: 23193181 DOI: 10.2337/db12-0975]
- 75 Méndez-del Villar M, González-Ortiz M, Martínez-Abundis E, Pérez-Rubio KG, Lizárraga-Valdez R. Effect of resveratrol administration on metabolic syndrome, insulin sensitivity, and insulin secretion. *Metab Syndr Relat Disord* 2014; **12**: 497-501 [PMID: 25137036 DOI: 10.1089/met.2014.0082]
- 76 la Porte C, Voduc N, Zhang G, Seguin I, Tardiff D, Singhal N, Cameron DW. Steady-State pharmacokinetics and tolerability of trans-resveratrol 2000 mg twice daily with food, quercetin and alcohol (ethanol) in healthy human subjects. *Clin Pharmacokinet* 2010; **49**: 449-454 [PMID: 20528005 DOI: 10.2165/11531820-00000000-00000]
- 77 Chow HH, Garland LL, Hsu CH, Vining DR, Chew WM, Miller JA, Perloff M, Crowell JA, Alberts DS. Resveratrol modulates drug- and carcinogen-metabolizing enzymes in a healthy volunteer study. *Cancer Prev Res (Phila)* 2010; **3**: 1168-1175 [PMID: 20716633 DOI: 10.1158/1940-6207.CAPR-09-0155]
- 78 Liu J. Pharmacology of oleanolic acid and ursolic acid. *J Ethnopharmacol* 1995; **49**: 57-68 [PMID: 8847885 DOI: 10.1016/0378-8741(95)90032-2]
- 79 Liu J. Oleanolic acid and ursolic acid: research perspectives. *J Ethnopharmacol* 2005; **100**: 92-94 [PMID: 15994040 DOI: 10.1016/j.jep.2005.05.024]
- 80 Checker R, Sandur SK, Sharma D, Patwardhan RS, Jayakumar S, Kohli V, Sethi G, Aggarwal BB, Sainis KB. Potent anti-inflammatory activity of ursolic acid, a triterpenoid antioxidant, is mediated through suppression of NF- $\kappa$ B, AP-1 and NF-AT. *PLoS One* 2012; **7**: e31318 [PMID: 22363615 DOI: 10.1371/journal.pone.0031318]
- 81 Ikeda Y, Murakami A, Ohgashi H. Ursolic acid: an anti- and pro-inflammatory triterpenoid. *Mol Nutr Food Res* 2008; **52**: 26-42 [PMID: 18203131 DOI: 10.1002/mnfr.200700389]
- 82 Kowalski R. Studies of selected plant raw materials as alternative sources of triterpenes of oleanolic and ursolic acid types. *J Agric Food Chem* 2007; **55**: 656-662 [PMID: 17263457 DOI: 10.1021/jf0625858]
- 83 Jäger S, Trojan H, Kopp T, Laszczyk MN, Scheffler A. Pentacyclic triterpene distribution in various plants - rich sources for a new group of multi-potent plant extracts. *Molecules* 2009; **14**: 2016-2031 [PMID: 19513002 DOI: 10.3390/molecules14062016]
- 84 Pubchem Open Chemistry Database. Bethesda: National Center for Biotechnology Information, U.S. National Library of Medicine; 2005. [accessed 2015 Jun 19]. Available from: URL: [https://pubchem.ncbi.nlm.nih.gov/search/#collection=compounds&query\\_type=text&query=Ursolic acid](https://pubchem.ncbi.nlm.nih.gov/search/#collection=compounds&query_type=text&query=Ursolic acid)
- 85 Ahmed Z, Ali D, Malik A. Structure determination of ursene-type triterpenes by NMR techniques. *Magn Reson Chem* 2006; **44**: 717-719 [PMID: 16607673 DOI: 10.1002/mrc.1803]
- 86 Zhang W, Hong D, Zhou Y, Zhang Y, Shen Q, Li JY, Hu LH, Li J. Ursolic acid and its derivative inhibit protein tyrosine phosphatase 1B, enhancing insulin receptor phosphorylation and stimulating glucose uptake. *Biochim Biophys Acta* 2006; **1760**: 1505-1512 [PMID: 16828971 DOI: 10.1016/j.bbagen.2006.05.009]
- 87 Na M, Yang S, He L, Oh H, Kim BS, Oh WK, Kim BY, Ahn JS. Inhibition of protein tyrosine phosphatase 1B by ursane-type triterpenes isolated from *Symplocos paniculata*. *Planta Med* 2006; **72**: 261-263 [PMID: 16534732 DOI: 10.1055/s-2005-873194]
- 88 He Y, Li W, Li Y, Zhang S, Wang Y, Sun C. Ursolic acid increases glucose uptake through the PI3K signaling pathway in adipocytes. *PLoS One* 2014; **9**: e110711 [PMID: 25329874 DOI: 10.1371/journal.pone.0110711]
- 89 Ali H, Houghton PJ, Soumyanath A. alpha-Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to *Phyllanthus amarus*. *J Ethnopharmacol* 2006; **107**: 449-455 [PMID: 16678367 DOI: 10.1016/j.jep.2006.04.004]
- 90 Lee EH, Popov SA, Lee JY, Shpatov AV, Kukina TP, Kang SW, Pan CH, Um BH, Jung SH. Inhibitory effect of ursolic acid derivatives on recombinant human aldose reductase. *Bioorg Khim* 2011; **37**: 637-644 [PMID: 22332359 DOI: 10.1134/S1068162011050050]
- 91 Jang SM, Kim MJ, Choi MS, Kwon EY, Lee MK. Inhibitory effects of ursolic acid on hepatic polyol pathway and glucose production in streptozotocin-induced diabetic mice. *Metabolism* 2010; **59**: 512-519 [PMID: 19846180 DOI: 10.1016/j.metabol.2009.07.040]
- 92 Wang ZH, Hsu CC, Huang CN, Yin MC. Anti-glycative effects of oleanolic acid and ursolic acid in kidney of diabetic mice. *Eur J Pharmacol* 2010; **628**: 255-260 [PMID: 19917277 DOI: 10.1016/j.ejphar.2009.11.019]
- 93 Li Y, Kang Z, Li S, Kong T, Liu X, Sun C. Ursolic acid stimulates lipolysis in primary-cultured rat adipocytes. *Mol Nutr Food Res* 2010; **54**: 1609-1617 [PMID: 20521271 DOI: 10.1002/mnfr.200900564]
- 94 Li D, Wang GL, Shan MY, Liu JH, Wang L, Zhu DZ. Effects of ursolic acid on c-Cbl-associated protein expression in 3T3-L1 adipocytes with insulin resistance. *Zhongxiyi Jiehe Xuebao* 2012; **10**: 886-893 [PMID: 22883405 DOI: 10.3736/jcim20120809]
- 95 Wang XH, Zhou SY, Qian ZZ, Zhang HL, Qiu LH, Song Z, Zhao J, Wang P, Hao XS, Wang HQ. Evaluation of toxicity and single-dose pharmacokinetics of intravenous ursolic acid liposomes in healthy adult volunteers and patients with advanced solid tumors. *Expert Opin Drug Metab Toxicol* 2013; **9**: 117-125 [PMID: 23134084 DOI: 10.1517/17425255.2013.738667]

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## Diabetic foot disease: From the evaluation of the “foot at risk” to the novel diabetic ulcer treatment modalities

Noha Amin, John Doupis

Noha Amin, Department of Internal Medicine (Diabetes and Metabolism Unit), Alexandria University, Alexandria 21526, Egypt

John Doupis, Iatriko Paleou Falirou Medical Center, Division of Diabetes and Clinical Research Center, 17562 Athens, Greece

John Doupis, Postgraduate Diabetes Education, Institute of Molecular and Experimental Medicine, Cardiff University School of Medicine, Cardiff, CF14 4XN, United Kingdom

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**Correspondence to:** John Doupis, MD, PhD, Iatriko Paleou Falirou Medical Center, Division of Diabetes and Clinical Research Center, 36 Areos st, Paleo Faliro, 17562 Athens, Greece. [john.doupis@joslin.harvard.edu](mailto:john.doupis@joslin.harvard.edu)  
Telephone: +30-210-9892300  
Fax: +30-210-9892300

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### Abstract

The burden of diabetic foot disease (DFD) is expected to increase in the future. The incidence of DFD is still rising due to the high prevalence of DFD predisposing factors. DFD is multifactorial in nature; however most of the diabetic foot amputations are preceded by foot ulceration. Diabetic peripheral neuropathy (DPN) is a major risk factor for foot ulceration. DPN leads to loss of protective sensation resulting in continuous unconscious traumas. Patient education and detection of high risk foot are essential for the prevention of foot ulceration and amputation. Proper assessment of the diabetic foot ulceration and appropriate management ensure better prognosis. Management is based on revascularization procedures, wound debridement, treatment of infection and ulcer offloading. Management and type of dressing applied are tailored according to the type of wound and the foot condition. The scope of this review paper is to describe the diabetic foot syndrome starting from the evaluation of the foot at risk for ulceration, up to the new treatment modalities.

**Key words:** Diabetes; Diabetic foot; Diabetic ulcer; Diabetic complications; Diabetic neuropathy; Diabetic macroangiopathy

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**Core tip:** Foot at risk evaluation is crucial to diabetic foot ulceration prevention. Diabetic foot ulcer treatment includes standard wound care procedures, as well as, other novel treatment modalities always as add on therapy.

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## INTRODUCTION

The diabetic foot syndrome or disease (DFD) includes several pathologies, mainly diabetic peripheral neuropathy and peripheral arterial disease which result in foot ulceration. Diabetic foot ulceration may ultimately lead to amputation, especially when wound infection or osteomyelitis are involved. Diabetic foot ulcer is defined as a full-thickness wound which is present at a level distal to the ankle in patients with diabetes<sup>[1,2]</sup>. Special categories like Charcot neuroarthropathy are also included in the DFD<sup>[3]</sup>. Patients with diabetic foot are also more likely to present with other diabetes-related complications such as nephropathy, retinopathy, ischemic heart disease and cerebrovascular disease<sup>[4]</sup>.

DFD is a common complication which is multifactorial in nature. A good understanding of its various predisposing risk factors would help in both prevention and treatment of this devastating medical condition. The present review paper attempts to address the major challenges and barriers for a better approach of the DFD.

## EPIDEMIOLOGY

DFD occurs in all types of diabetes showing higher prevalence among males and in patients more than 60 years old<sup>[3]</sup>. The burden of DFD is expected to rise in the future, giving that the prevalence of its predisposing factors - mainly the diabetic peripheral neuropathy and peripheral limb ischemia are continually increasing<sup>[5]</sup>. Epidemiological studies for the DFD incidence and prevalence, present various conduction difficulties mostly related to the diagnostic tests used and the population selection<sup>[6]</sup>.

The annual incidence of foot ulceration is estimated to be approximately 1%-4%<sup>[7,8]</sup>, and its prevalence ranges from 4% to 10%, whereas, the lifetime risk for the development of a diabetic foot ulcer in patients with diabetes ranges from 15% to as high as 25%<sup>[7,9]</sup>.

The presence of foot ulceration is considered to be the main precursor of a lower extremity amputation among patients with diabetes<sup>[10]</sup>. Apart from the diabetic peripheral neuropathy and the peripheral vascular disease, several other risk factors were identified such as, the limited joint mobility, the foot deformities and any previous ulceration or amputation at the same or contralateral limb. Other risk factors are related to the patient's general condition including; impaired visual acuity, older age, chronic renal disease, long duration of diabetes & sustained uncontrolled hyperglycemia<sup>[11-14]</sup>.

## DIABETIC NEUROPATHY

According to the International Consensus Group on



**Figure 1** A neuropathic ulcer in a patient with severe diabetic peripheral neuropathy.

Neuropathy; the diabetic neuropathy is defined as the detection of manifestations of peripheral nerve dysfunction in people with diabetes, after excluding other possible causes of peripheral neuropathy<sup>[15]</sup>. It is not an uncommon condition; in fact it is one of the most common long term complications of diabetes and the most common form of neuropathy in many parts of the world.

The presence of diabetic peripheral neuropathy, even with trivial trauma, is the initiating factor of the development of foot ulceration in patients with diabetes. It has been reported that the risk for diabetic foot ulceration increases by seven fold in patients with peripheral diabetic neuropathy<sup>[16,17]</sup>. It is also estimated that 45% to 60% of all ulcerations in patients with diabetes are mainly due to neuropathy, while 45% of the ulcers are due to combined neuropathic and ischemic factors (Figure 1)<sup>[12,16,18,19]</sup>.

### *Distal bilateral symmetrical neuropathy*

This form of neuropathy is the commonest presentation among patients with diabetes. It usually starts in the lower limbs while the upper limbs may be also involved too in some cases. It has a progressive course, starting distally and then proceeds proximally as the severity of nerve dysfunction increases. It usually presents in a glove and stocking pattern of abnormal sensations<sup>[20]</sup>.

The distal symmetrical diabetic neuropathy may present with different clinical symptoms. Patients may describe it as symptoms of unpleasant sensations such as tingling, burning, prickling, electric shocks, lancinating pain, hyperalgesia (exaggerated perception of pain on application of a painful stimulus) or even allodynia (contact pain or pain perception due to a non-painful stimulus). Some patients may report abnormal cold or hot feelings in their feet or persistent painful cramp-like sensations even at rest<sup>[21]</sup>. It is worth mentioning that most of the patients may be completely asymptomatic and unaware of having peripheral neuropathy. Patients may present with diabetic foot ulceration even without any preceding neuropathic complaints<sup>[12]</sup>.

Although the sensory nerve fibers are the most

commonly affected fibers, motor nerve fibers are sometimes affected too, leading to muscle denervation. During the early course of the disease, the muscle power is preserved except mild muscle weakness in the toe extensors. As the disease progresses muscle weakness becomes more generalized affecting small muscles in both feet and hands. This muscle wasting can result in altering the normal foot dynamics and pressure distribution. Wasting and atrophy of small muscles in the foot lead to loss of joint stability and the development of foot deformities. Foot deformities may take several forms such as equinus or varus deformity, hammer toes, cocked-up toes and flat foot changes. These changes lead to pressure distribution disturbance, increased shear stress and friction, ultimately leading in foot ulceration<sup>[22-25]</sup>. Diabetic peripheral neuropathy is also characterized by the loss of the deep sensation, such as vibration perception and proprioception which in severe cases might lead to sensory ataxia and a positive Romberg's sign. Deep tendon reflexes are usually impaired or lost starting with ankle reflex and progressing proximally to the knee reflex<sup>[21]</sup>.

Additionally diabetic autonomic neuropathy may result in sudomotor dysfunction leading to abnormal sweating and dry skin with cracking and fissuring facilitating the bacterial infection of the foot<sup>[26]</sup>. Autonomic neuropathy is also associated with thermoregulatory dysfunction and abnormal tissue perfusion. Autonomic neuropathy is also in many cases associated with an unexplained foot edema which is resistant to diuretics. This edema results from shunt opening and hyperkinetic circulation, further adding to the risk of foot ulceration<sup>[27]</sup>.

### **Diagnosis of the diabetic peripheral neuropathy**

Diabetic peripheral neuropathy is diagnosed through careful patient history review and physical examination of the feet. Using the combination of patient's neuro-pathic symptoms, clinical signs and electrodiagnostic tests would be the best predictor for diabetic peripheral neuropathy<sup>[28]</sup>.

**Symptom scores:** Various verbal descriptive scales and simple visual analog scales are used in clinical practice to assess and follow up the neuropathic symptoms in response to treatment<sup>[29,30]</sup>. Symptom scores are used for the evaluation of painful diabetic neuropathy. The most widely used ones are: The Neuropathy Symptom Score (NSS), which is widely used in clinical practice has shown high validity and sensitivity<sup>[31-34]</sup>. Several other adaptations are also available such as the Neuropathy Symptom Profile, the modified NSS scores of Veves and Young, the Michigan Neuropathy Screening Instrument, and Diabetic NSS<sup>[17,35-38]</sup>.

**Semmes-Weinstein monofilament:** Semmes-Weinstein monofilament is a widely used tool for the assessment of the diabetic peripheral neuropath in every day clinical practice<sup>[39,40]</sup>. It assesses the protective

ability (evaluates A-beta fibers, determining the patient's threshold for light touch and pressure) of the foot through the application of gentle pressure to the handle until the nylon filament is buckled for 2 s. Many different sizes of filaments are available with the 10-g pressure monofilament (5.07 monofilament) to be the most commonly used for pressure sensation evaluation. Around 90% of the patients with insensate diabetic foot could be identified on testing four planter sites (great toe and the base of first, third and fifth metatarsals)<sup>[41]</sup>. Monofilament test has shown a sensitivity of (66%-91%) in detection of diabetic patients at high risk for foot ulceration in several studies<sup>[18,42,43]</sup>. The monofilament test is a quick and painless method, easily acceptable from the patient, easy to administer by the physicians, portable and inexpensive<sup>[40]</sup>.

**Vibration perception:** The impairment of vibration perception is usually one of the earliest signs of peripheral diabetic neuropathy. Vibration assessment evaluates the large diameter fibers (A-beta fibers). There are several ways for examining the vibration perception threshold (VPT), including: (1) 128 Hz tuning fork: It assesses the vibration perception through application on distal bony prominences of the great toe bilaterally and proceeds proximally on other bony prominences such as the medial malleolus and tibial tuberosity if impairment is noted. Tuning fork gives around 53% sensitivity and there is evidence suggesting that compared to the monofilament test, tuning fork is less predictive for development of foot ulceration<sup>[18]</sup>; (2) graduated rydel-seiffer tuning fork: The graduated tuning fork depends on optical visual illusion. The fork has 0-8 graded scale, where the examiner can detect the point of vibration impairment or disappearance<sup>[44]</sup>. Application of the graduated tuning fork detects the presence of vibration perception impairment and the intensity of this impairment. The reduction of vibration perception to less than 4/8 was present in 95% of diabetic foot ulcerations due to peripheral neuropathy<sup>[44,45]</sup>; (3) neurothesiometer; and (4) biothesiometer.

The neurothesiometer and biothesiometer are electronic devices. They depend on sending vibrations of various strengths through a probe applied to the bony prominence of the great toe. The vibrations are measured in volts per micrometer. As the VPT of the patient increases, the risk for diabetic foot ulceration due to neuropathy increases. A vibration threshold of more than 25 V has been reported to have a sensitivity of 83%. The risk of ulceration in the group of patients with sensitivity between 25 and 33 V was increased by eight times compared to twenty-fold increase associated with values of more than 42 V<sup>[46,47]</sup>.

**Nerve conduction studies:** Nerve electrophysiologic conduction studies are not routinely used in clinical practice for diagnosis of diabetic neuropathy. They

are objective, non-invasive, highly reliable parametric measures which are useful in monitoring the progression of diabetic peripheral neuropathy especially in asymptomatic patients<sup>[48]</sup>. They are also extremely useful in atypical presentations of neuropathy and in superimposed forms of mononeuropathies<sup>[49]</sup>. Electromyography and nerve conduction studies such as NCV and F waves can detect the type of nerve injury, extend, symmetry and severity of the lesion<sup>[50]</sup>.

**Other methods of assessment:** (1) Nerve biopsy: It is an invasive procedure used for diagnosis of peripheral neuropathy and atypical presentations in patients with diabetes, usually using sural nerve biopsy<sup>[51]</sup>. Nerve biopsy has many complications such as postoperative pain at the site of nerve biopsy, parasthesia, allodynia and sensory disturbances at the sites of nerve distribution especially in patients with diabetes<sup>[52]</sup>. Assessment of efficacy of treatment and disease progression can be determined depending on morphological parameters such as axonal atrophy, density of myelinated fibres and axo-glial dysjunction<sup>[53,54]</sup>; (2) Skin biopsy: It is another less invasive technique alternative to nerve biopsy for studying small nerve fibers using a 3-mm skin biopsy in clinical studies<sup>[55]</sup>. Several neuronal markers are used to immunostain skin nerves, such as neurone-specific enolase and somatostatin. The best cytoplasmic axonal marker has been proposed is the protein gene product-9.5. Formalin-fixed frozen sections are used in clinical research to visualize and assess the density of intraepidermal nerve fibers<sup>[56,57]</sup>.

### Is there a role for validated scores?

Validated scores are available in order to standardize the clinical assessment for the severity of symptoms and the grade of neuropathic impairment. The validated scores include; Neuropathy Disability Score for neuropathic deficits (impairments), NSS for neuropathic symptoms<sup>[17]</sup> and the Michigan Neuropathy Screening Instrument<sup>[36]</sup>. According to the Neuropathy Disability Score and the NSS, minimum criteria required for the clinical diagnosis of neuropathy are: (1) the presence of moderate signs of neuropathy in the presence or absence of symptoms; (2) the detection of mild signs in the presence of moderate symptoms.

## PERIPHERAL ARTERIAL DISEASE

Peripheral arterial disease (PAD) is one of the multifactorial causes leading to the diabetic foot disease. The presence of PAD alters the normal body response to foot ulcerations and leads to persistent non-healing foot ulcers, when there is an increased need for blood supply. PAD leads to progression of infection, increases tissue break down and insufficient delivery of oxygen, nutrition and antibiotics. All these factors further contribute to a potential foot amputation<sup>[58]</sup>.

PAD shows higher prevalence among patients with



**Figure 2** Gangrene in a patient with type 2 diabetes and severe peripheral arterial disease.

diabetes than the general population. PAD among patients with diabetes is characterized by onset at an earlier age, increased severity, a more rapid progression and equal sex distribution<sup>[59]</sup>. About 20% of patients having symptomatic PAD had diabetes as reported by the Framingham Heart Study<sup>[60]</sup>. In patients with diabetes, the risk of PAD is increased by advanced age, duration of diabetes, uncontrolled hyperglycemia and the association with diabetic peripheral neuropathy. The presence of diabetes is mostly associated with below knee PAD such as tibial, popliteal and femoral arterial affection, in contrast to more proximal PAD in the aorto-iliac vessels associated with other risk factors such as hypertension and smoking<sup>[61]</sup>.

PAD is characterized by the presence of intermittent claudication, which is defined as cramping or aching pains usually in the calf muscles, but can also be present in thighs or the buttocks. Intermittent claudication is aggravated by walking exercise that it forces the patient to stop walking and relieved by rest. In severe cases of PAD, pain may be present even at rest, limb may show gangrenous changes, tissue loss; which is known as critical limb ischemia (Figure 2)<sup>[62]</sup>.

### Diagnosis of PAD

**The ankle-brachial index:** The ankle-brachial index (ABI) is a simple bed-side screening tool for the presence of PAD. PAD simply depends on the calculation of the ratio between the systolic pressure of the ankle arteries and the systolic pressure at the brachial arteries<sup>[63]</sup>. ABI is an inexpensive method that can assess the severity of PAD as it usually correlates with the patient's reported symptoms and functional status. The normal range of ABI is between 0.9-1.3, falsely elevated values of ABI can result in cases of calcified, non-compressible arteries. Thus the ABI method may lead to underestimation of the severity of the disease in patients with diabetes<sup>[64]</sup>.

**The toe-brachial index:** The toe-brachial index is calculated similar to the ABI, where the systolic pressure is measured using a small cuff and a Doppler probe.



Measuring the toe-brachial index is helpful especially in cases of ABI values more than 1.30, as the small arteries of the lower limb are less likely to be calcified. A toe-brachial index lower than 0.70 is diagnostic for PAD<sup>[65]</sup>.

**Segmental limb pressure assessment and pulse volume recordings:** The technique depends on plethysmographic cuffs situated over the brachial arteries and different points on the lower limb. The extent and location of PAD can be detected from segmental systolic pressure assessment using a Doppler probe<sup>[66]</sup>.

**Ultrasound velocity spectroscopy and imaging:** The normal arterial Doppler velocity shows a triphasic signal. When an arterial obstruction is present proximal to the probe, there is loss of the normal reversed flow component on transforming the waveform associated with decreased amplitude, attenuation of all parts of the spectrum and delayed upstroke<sup>[67]</sup>.

Duplex ultrasound depends on combining the B-mode and the pulsed Doppler ultrasound to assess arterial flow and localized velocity information at stenotic sites. Duplex ultrasonography is widely used nowadays detecting with high sensitivity and specificity the arterial patency and extends of obstruction<sup>[68]</sup>. Duplex ultrasound has certain limitations mainly difficulty in identifying close multiple separate lesions, some difficulty when assessing infrapopliteal, common and external iliac arteries<sup>[69,70]</sup>.

**Transcutaneous oximetry and laser - doppler flowmetry:** These techniques are used mainly to assess cutaneous blood flow. Cutaneous blood flow is usually normal until late stages of proximal arterial ischemia of the atherosclerotic type, thus, this type of vascular evaluation is not used in every day practice<sup>[64]</sup>.

Magnetic resonance angiography (MRA)<sup>[71,72]</sup>.

**Computed tomographic angiography:** CTA is superior to MRA as it can detect the presence of calcification, which is advantageous on planning revascularization strategies. The ACC/AHA guidelines recommend CTA on deciding the revascularization techniques in cases of PAD, offering faster image than MRA<sup>[63]</sup>.

**Contrast angiography:** Although it is the gold standard for the diagnosis of PAD, is rarely required as a diagnostic tool due to the risks associated with invasive procedures. Computer-enhanced digital subtraction angiography can be useful in patients who present with localized stenosis so as to minimize the amount of contrast material injected and for better image resolution<sup>[63]</sup>.

Diabetic foot ulcers.

## CLASSIFICATION

The presence of diabetic foot ulceration is the main leading risk for amputation in patients with diabetes. Proper assessment and classification of a diabetic foot ulcer is an essential part for the management of the diabetic foot. A prompt and adequate ulcer treatment may lead to foot amputation prevention, preserving the life quality of the patient.

Several classifications have been proposed for the categorization of diabetic foot ulcers. The most important ones are described below.

### *Wagner-Meggitt classification*<sup>[73]</sup>

It is one of the earliest and most widely used classifications. It classifies the diabetic foot ulceration depending on how deep the wound is, includes 6 grades: (1) Grade 0: The skin is intact; (2) Grade 1: Presence of ulcer which is superficial; (3) Grade 2: Presence of ulcer which is deep; (4) Grade 3: Deep ulcer with abscess, bone involvement or osteomyelitis; (5) Grade 4: Gangrene in the forefoot; and (6) Grade 5: Whole foot gangrene.

Wagner-Meggitt classification has shown several disadvantages: (1) cannot address all patterns of diabetic foot ulcerations and infections; (2) the presence of infection is addressed in only one stage, thus, the superficial ulcers if infected or ischemic are not properly categorized in this system; and (3) this system does not properly assess the presence of peripheral ischemia in categorization of foot ulcers.

### *The university of texas system*<sup>[73]</sup>

It classifies diabetic foot ulcers into 4 grades (0-4) according to their depth, and then stages every grade of them according to the presence or absence of infection and ischemia (A-D).

The University of Texas Classification has been validated and has prognostic advantages as it included both infection and ischemia but showed some difficulty in application in day to day practice.

### *The SAD classification*<sup>[74]</sup>

This classification grades the diabetic foot ulceration according to five ulcer features (size, depth, sepsis, arteriopathy, and denervation) on a 4-point scale (0-3).

SAD classification differs from the other earlier systems by considering both size of ulcer and the presence of neuropathy. It has been validated by demonstrating differences between baselines variable and clinical outcome. Its major drawback is the complexity in practical use.

### *The pedis classification*<sup>[75]</sup>

The PEDIS system has been proposed by the International Working Group on the Diabetic Foot. This system grades the wound based on five features: (1) perfusion (arterial blood supply); (2) extent (area of the ulcer); (3)



depth of the wound; (4) presence of infection; and (5) sensation.

### ***The Infectious Diseases Society of America guidelines***<sup>[76]</sup>

These guidelines sub classified the infected diabetic foot into three categories: (1) mild: Involvement is restricted to skin and subcutaneous tissues; (2) moderate: Involvement is more extensive or affecting deeper tissues; and (3) Severe: Diabetic foot ulceration is accompanied by systemic signs of infection or metabolic decompensation.

## **PRINCIPLES OF DIABETIC FOOT ULCERS MANAGEMENT**

### ***Wound debridement***

Debridement of diabetic foot ulcers is an important initial step in the management of the wound. Several benefits can result from proper debridement including the removal of the necrotic and non-viable tissues and keeping a healthy granular wound bed. One should be careful on the assessment of the ulcer if ischemia is suspected. A revascularization intervention may be necessary before a debridement is performed. Debridement is also stimulating the release of growth factors to promote advancing healing edges<sup>[77,78]</sup>. Various methods are used for wound debridement.

**Surgical debridement:** It is the gold standard method in diabetic foot ulceration. To obtain optimal results, healthy tissue loss should be minimized, foot function should be preserved, and deformities which can precipitate recurrence of ulcers should be prevented. Surgical debridement is typically done for ulcers with large amount of necrotic and non-viable tissues. Debridement is performed using a scalpel blade with the tip pointed in a 45-degree angle or a tissue nipper to remove all necrotic and non-viable tissues until a bleeding healthy base is obtained<sup>[79]</sup>.

**Enzymatic debridement:** Enzymatic debridement is based on the application of topical agents on the ulcer. These agents are usually applied once daily. Their action is based on the necrotic tissue degradation using proteolytic digestive enzymes such as streptokinases, trypsin, papain, fibrinolysin-DNase, collagenase, papain-urea and streptodornase. Data from clinical studies have shown conflicting results about the efficacy of these topical agents, thus, their additional benefits to standard wound care remains unclear. Putting into consideration the need of long time application, as well as, the high cost, their use is usually limited to slowly soften large eschars or debridement of some decubitus ulcerations in sensate limbs. In order to improve efficacy of these agents, a scalpel blade is applied to crosshatch eschars<sup>[80]</sup>.

**Mechanical debridement:** Although it is a simple and

an inexpensive tool, it can remove both viable and also non-viable tissues leading to pain in sensate foot. The wet gauze dressing is applied to the wound bed and then kept to dry. The necrotic debris embedded in the gauze is mechanically stripped from the wound bed on gauze removal<sup>[81]</sup>.

**Biological debridement (Maggot therapy):** Recently the use of Maggot therapy has re-emerged showing benefits in necrotic tissue debridement, decreasing bacterial load and stimulation of wound healing. Several studies showed the efficacy of Maggot therapy<sup>[82-84]</sup>.

**Other modalities:** Ongoing research is evaluating other methods such as low energy ultrasound mist for the debridement of diabetic foot ulcers<sup>[84]</sup>.

### ***Pressure offloading***

The pressure offloading relieves abnormal pressure applied to the ulcer promoting the wound healing. Several methods have been applied for offloading including; total contact casting (TCC), short leg walkers, half shoes and felted foam dressings. TCC is based on a well-molded plaster cast, resulting in equal pressure distribution to the whole lower limb. This method is very effective with a good wound healing rate when applied properly and changed at least weekly. Although it is an effective method it has significant disadvantages which may limit their use and the choice of other alternatives. Disadvantages of TCC include; time and skill required to be applied properly, secondary skin irritation and ulceration resulting from the cast applied, and impossible daily assessment of the wound<sup>[85,86]</sup>.

Other alternative to TCC is the Scotch-cast boot with a cast sandal to increase mobility and at the same time ensure ulcer relief from pressure. Commercial devices such as the short leg walker and half shoe are readily available, they are preferred by the patients with a better acceptance, simple, easy to apply and inexpensive. Their major disadvantage is that patients can remove them thus cannot ensure patient's compliance with less significant pressure relieving results compared to the TCC<sup>[87]</sup>.

Felted foam dressings allow a customized pressure relief through a felt-foam pad with an opening over the ulceration through which wound assessment and care can be done. The felted foam is used in combination with half-shoe or surgical shoe and when used properly and changed every 10-14 d, has shown more efficacy compared to half-shoe or short walkers alone<sup>[88]</sup>.

### ***Treatment of infection***

The presence of infection is a common finding in diabetic foot ulcers which act as an entry route for pathogens. Infections must be diagnosed and treated promptly and adequately as they may rapidly progress to a limb-threatening condition<sup>[78]</sup>. Also high levels of bacteria can delay or even prevent wound healing and impede

surgical closure of diabetic ulcers<sup>[89]</sup>.

**Diagnosis of diabetic foot ulcer infection:** Diagnosis of infection is based initially on clinical signs such as redness, temperature, pain, tenderness, edema and the presence of suspected discharge. On clinical suspicion of infection, properly taken cultures from the wound area may be helpful in proper antibiotic treatment selection. It is important to point out that uninfected ulcers is not necessary to be cultured as the results will only indicate the colonizing flora. The most common pathogens in diabetic foot ulcers are aerobic gram positive cocci and gram negative bacteria. Anaerobic organisms are frequently isolated too<sup>[78]</sup>. Staphylococcus and streptococci are the most frequently causative agents for non-threatening limb infections while limb-threatening infections are mostly polymicrobial in nature<sup>[90]</sup>.

The use of antibiotics in infected diabetic foot ulcer should be carefully applied, in order to be assured that the patient will receive the appropriate antibiotic therapy, for an adequate period of time, along with wound debridement and drainage<sup>[90]</sup>.

#### Diabetic foot care

To be able to provide an effective plan for diabetic foot syndrome prevention and treatment, a multidisciplinary team approach is required.

This multidisciplinary team approach includes<sup>[10,91]</sup>: (1) diabetologist/Endocrinologist to optimize the metabolic control for patients with diabetes; (2) diabetes educator and a qualified nurse: To provide special education and assurance; (3) podiatrist who would guide the patient to prevent diabetic foot lesions and provide appropriate treatment; (4) vascular surgeon to assess the vascularity of the lower limbs and provide interventional management whenever required; (5) orthotist: Help in choosing the appropriate foot wear or custom foot wear to allow adequate pressure distribution and thus rapid wound healing; (6) infection disease specialist: For appropriate choice of antibiotics regimen based on culture results; and (7) nutritionist consultation to help in adequate glycemic control, weight loss and also wound healing.

## MODERN WOUND CARE MODALITIES

In the recent years, apart from the standard wound care, new diabetic ulcer treatment modalities have been developed<sup>[92,93]</sup>.

#### Wound dressings

The ideal wound dressing<sup>[94]</sup>: (1) should be sterile and does not contaminate the ulcer with foreign particles; (2) should be readily available, easy to use and cost effective; (3) should keep a moist environment for adequate wound healing; (4) should be able to absorb excess exudates from the wound; (5) should not

adhere to the wound floor, also should be non-allergic and non-toxic; (6) should be able to protect the wound from microorganisms and also provides mechanical protection; and (7) should maintain adequate tissue gaseous exchange and control wound odor.

#### Topical agents

**Wet to dry dressing (simple saline dressing):** Wet to dry dressing is included in standard wound care and is considered a method for mechanical debridement, since it presents a good debriding effect in removal of the necrotic tissue and wound preparation<sup>[95]</sup>. In order to minimize irritation and discomfort, adequate moistening of the dressings with normal saline is done when treating granulating wound tissues to avoid trauma and bleeding<sup>[96]</sup>.

**Local antibacterial agents:** Antibacterial agents can be used alone or in combination with other dressings except for dry necrotic ulcers. For effective anaerobic coverage, metronidazole gel is used and maintains a moist environment for wound healing<sup>[97,98]</sup>. Several antibiotics have effective antibacterial action on topical application such as Neomycin, Gentamycin, and Mupirocin. Silver dressings and polyherbal topical preparations have shown good antibacterial action<sup>[99]</sup>. For effective antibacterial action against Pseudomonas, other gram-negative bacilli, and beta hemolytic streptococci wound infections Sisomycin and acetic acid can be used. Special precautions should be considered when using povidone iodine solution dressings, iodine has been found to be toxic at high concentrations to bacteria and fungi as well as human cells<sup>[100,101]</sup>.

**Tulle dressings:** Tulle dressings are used mainly for skin grafts and superficial wounds. They can be safely used in granulating and epithelializing tissues as they are impregnated with paraffin, having low dressing adherence properties<sup>[102]</sup>. Evidence from several previous studies have shown better and faster re-epithelialization rates compared to dry dressing<sup>[102-104]</sup>.

**Hydrogel dressings:** Hydrogel dressings are considered the best choice for dry wounds with necrotic eschar. Hydrogels provide fluid and good hydration to dry and slough wounds. Although they are very good at absorbing exudates, they should be avoided in diabetic foot planter ulcers as they may cause maceration of the skin surrounding the wound<sup>[105-107]</sup>.

**Polyurethane films:** Polyurethane films are transparent films coated with a water-proof adhesive dressing. They provide permeable films allowing diffusion of gases and vapor thus an adequately moist wound environment. They have the advantage of being transparent, thus can monitor the wound progression. They also can be used for low exudates wounds, but they may cause maceration of the skin surrounding the

wound<sup>[108]</sup>.

**Polyurethane foam:** Polyurethane foam is highly used in diabetic foot ulcers. It can absorb large amounts of exudates in a non-adherent nature thus does not cause wound sloughing or trauma on removal. They maintain moisture environment thus allow proper preparation of wound bed and promotes better wound healing<sup>[109]</sup>.

**Alginate dressings:** Two forms of alginate dressings are available; calcium alginate and calcium sodium alginate. Alginate dressings can absorb large amounts of exudates up to 20 times their weight as shown by several clinical studies<sup>[110]</sup>.

**Honey-impregnated dressings:** The anti-inflammatory and anti-microbial actions have been shown *in vitro* studies but further studies are required to support strong evidence *in vivo*<sup>[111,112]</sup>.

### Growth factors

Growth factors have shown promising results in diabetic ulcer healing. Growth factors stimulate angiogenesis, cellular proliferation and migration, together with promoting enzymatic production. Several growth factors have been studied including; platelet-derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor (TGF)- $\beta$ , TGF- $\alpha$  and insulin-like growth factor..., etc.<sup>[113]</sup>. A recombinant human (rh)-PDGF dressing is available for diabetic foot lesions when added to conventional<sup>[114]</sup>. EGF in the form of local injections of rh-EGF showed favorable results in neuropathic vs ischemic ulceration<sup>[115]</sup>.

The Food and Drug Administration (FDA) announced some warnings regarding the use of a recombinant human platelet-derived growth factor, which contains becaplermin. In both clinical studies and post marketing users, becaplermin was associated with malignancies distant from the application site. Also increased mortality rate from systemic malignancies was reported on using 3 or more tubes of becaplermin gel. Topical enzymes: Several prepared ointments containing enzymes such as fibrinolysin, collagenase or papain have been used in enzymatic debridement of the sloughy tissues and promoting granulation tissue formation. Papain-urea has shown better enzymatic debridement effect when compared to collagenase<sup>[116]</sup>.

**Vacuum-assisted closure:** Vacuum-assisted devices have shown efficacy in exudates removal and edema reduction. Ideally a pressure of 125 mmHg can generate a negative topical pressure over the diabetic foot wound. It has the advantage of leaving the wound surface moist. It has several limitations; it is contraindicated in cases of osteomyelitis, ischemia, deep tissues exposure such as tendons, bones and blood vessels, presence of necrotic tissues and fistulas<sup>[117]</sup>. Vacuum-assisted devices are also effective in promoting closure and wound healing in patients with treated infections

and treated osteomyelitis<sup>[118,119]</sup>.

### Hyperbaric oxygen therapy, Do we have evidence?

A systemic treatment where oxygen is breathed but at a higher pressure than the local atmospheric pressure<sup>[120]</sup>. HBOT has shown increased healing rates of diabetic foot ulcers, however it still controversial whether it can be used as adjuvant treatment or not<sup>[121]</sup>. Hyperbaric oxygen therapy (HBOT) have the advantage of reduction of tissue hypoxia, edema, increase angiogenesis and erythrocytes deformability, antimicrobial effects and increase fibroblastic activity<sup>[122-124]</sup>. HBOT is approved as an adjunctive treatment to be used in chronic non-healing ulcers by the Undersea and Hyperbaric Medical Society<sup>[125]</sup>. The European Committee for Hyperbaric Medicine has set a type 2 recommendation for the use of HBOT in the management of diabetic foot ulcers including patients with ischemic wounds without a surgically treatable arterial lesion or as a complement after vascular surgery, in presence of non-healing wounds<sup>[126]</sup>.

**The role of stem cell therapy in PAD:** It is worth mentioning that our skeletal muscles have a regenerative capacity as they have multipotential and progenitor cells. In cases of critical limb peripheral arterial disease, the transplantation of progenitor cells- derived from bone marrow- has beneficial effects on angiogenesis and ulcer healing as shown in phase I and II studies. The role therapeutic angiogenesis is a promising and a safe method for management of PAD and limb salvage<sup>[127]</sup>.

## CONCLUSION

Concluding, diabetic foot ulceration is generally preventable. The first step in ulcer prevention is the careful screening for foot problems and detection of patients at high risk. More research is still required to improve the diagnosis of conditions leading to foot ulceration. Diversity in the diagnostic criteria and the lack of cut off hinders the standardization of management plans. Multi-disciplinary team approach is required to effectively manage the different aspects of diabetic foot syndrome. Standard wound care is recommended, while modern treatment modalities have shown some promising results in recent studies.

## REFERENCES

1. **Reiber GE, Ledoux WR.** Epidemiology of diabetic foot ulcers and amputations: Evidence for prevention. In: Williams R, Herman W, Kinmonth AL, Wareham NJ. The evidence base for diabetes care. Chichester; Hoboken (NJ): John Wiley & Sons, Ltd, 2003: 641-665 [DOI: 10.1002/0470846585.ch28]
2. **Apelqvist J, Bakker K, van Houtum WH, Nabuurs-Franssen MH, Schaper NC.** International consensus and practical guidelines on the management and the prevention of the diabetic foot. International Working Group on the Diabetic Foot. *Diabetes Metab Res Rev* 2000; **16** Suppl 1: S84-S92 [PMID: 11054895 DOI: 10.1002/1520-7560(200009/10)16:1]



- 3 **Rathur HM**, Boulton AJ. The diabetic foot. *Clin Dermatol* 2007; **25**: 109-120 [PMID: 17276208 DOI: 10.1016/j.clindermatol.2006.09.015]
- 4 **Khanolkar MP**, Bain SC, Stephens JW. The diabetic foot. *QJM* 2008; **101**: 685-695 [PMID: 18353793 DOI: 10.1093/qjmed/hcn027]
- 5 **UK Prospective Diabetes Study (UKPDS) Group**. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 1998; **352**: 837-853 [PMID: 9742976 DOI: 10.1016/S0140-6736(98)07019-6]
- 6 **Williams DR**, Airey M. The size of the problem: Epidemiological and economical aspects of the diabetic foot. In: Boulton AJM, Connor H and Cavanagh PR. The foot in diabetes. Chichester England; New York: Wiley, 2000: 3-17
- 7 **Bartus CL**, Margolis DJ. Reducing the incidence of foot ulceration and amputation in diabetes. *Curr Diab Rep* 2004; **4**: 413-418 [PMID: 15539004 DOI: 10.1007/s11892-004-0049-x]
- 8 **Armstrong DG**, Lipsky BA. Advances in the treatment of diabetic foot infections. *Diabetes Technol Ther* 2004; **6**: 167-177 [PMID: 15117583 DOI: 10.1089/152091504773731357]
- 9 **Boulton AJ**, Vileikyte L, Ragnarson-Tennvall G, Apelqvist J. The global burden of diabetic foot disease. *Lancet* 2005; **366**: 1719-1724 [PMID: 16291066 DOI: 10.1016/S0140-6736(05)67698-2]
- 10 **American Diabetes Association**. Consensus Development Conference on Diabetic Foot Wound Care: 7-8 April 1999, Boston, Massachusetts. American Diabetes Association. *Diabetes Care* 1999; **22**: 1354-1360 [PMID: 10480782 DOI: 10.2337/diacare.22.8.1354]
- 11 **Frykberg RG**. Diabetic foot ulcers: pathogenesis and management. *Am Fam Physician* 2002; **66**: 1655-1662 [PMID: 12449264]
- 12 **Boulton AJ**, Kirsner RS, Vileikyte L. Clinical practice. Neuropathic diabetic foot ulcers. *N Engl J Med* 2004; **351**: 48-55 [PMID: 15229307 DOI: 10.1056/NEJMc032966]
- 13 **Boulton AJ**. The diabetic foot: from art to science. The 18th Camillo Golgi lecture. *Diabetologia* 2004; **47**: 1343-1353 [PMID: 15309286 DOI: 10.1007/s00125-004-1463-y]
- 14 **Frykberg RG**, Zgonis T, Armstrong DG, Driver VR, Giurini JM, Kravitz SR, Landsman AS, Lavery LA, Moore JC, Schuberth JM, Wukich DK, Andersen C, Vanore JV. Diabetic foot disorders. A clinical practice guideline (2006 revision). *J Foot Ankle Surg* 2006; **45**: S1-S66 [PMID: 17280936 DOI: 10.1016/S1067-2516(07)60001-5]
- 15 **Katsilambros N**. Who is the patient at risk for foot ulceration? In: Katsilambros N, Dounis E, Tzapogas P and Tentolouris N Atlas of the diabetic foot. Chichester; Hoboken, NJ: Wiley, 2003: 1-21 [DOI: 10.1002/047086138x.ch1]
- 16 **Reiber GE**, Vileikyte L, Boyko EJ, del Aguila M, Smith DG, Lavery LA, Boulton AJ. Causal pathways for incident lower-extremity ulcers in patients with diabetes from two settings. *Diabetes Care* 1999; **22**: 157-162 [PMID: 10333919 DOI: 10.2337/diacare.22.1.157]
- 17 **Young MJ**, Boulton AJ, MacLeod AF, Williams DR, Sonksen PH. A multicentre study of the prevalence of diabetic peripheral neuropathy in the United Kingdom hospital clinic population. *Diabetologia* 1993; **36**: 150-154 [PMID: 8458529 DOI: 10.1007/BF00400697]
- 18 **Boyko EJ**, Ahroni JH, Stensel V, Forsberg RC, Davignon DR, Smith DG. A prospective study of risk factors for diabetic foot ulcer. The Seattle Diabetic Foot Study. *Diabetes Care* 1999; **22**: 1036-1042 [PMID: 10388963 DOI: 10.2337/diacare.22.7.1036]
- 19 **Abbott CA**, Carrington AL, Ashe H, Bath S, Every LC, Griffiths J, Hann AW, Hussein A, Jackson N, Johnson KE, Ryder CH, Torkington R, Van Ross ER, Whalley AM, Widdows P, Williamson S, Boulton AJ. The North-West Diabetes Foot Care Study: incidence of, and risk factors for, new diabetic foot ulceration in a community-based patient cohort. *Diabet Med* 2002; **19**: 377-384 [PMID: 12027925 DOI: 10.1046/j.1464-5491.2002.00698.x]
- 20 **Tesfaye S**. Diabetic neuropathy. In: Boulton AJM, Cavanagh PR and Rayman G The foot in diabetes. Chichester, England; Hoboken, NJ: Wiley, 2006: 30-40 [DOI: 10.1002/0470029374.ch3]
- 21 **Tesfaye S**. Clinical features of diabetic polyneuropathy. In: Veves A and Malik RA Diabetic neuropathy: Clinical management. Totowa, NJ: Humana Press, 2007: 243-257 [DOI: 10.1007/978-1-59745-311-0\_14]
- 22 **Hutton W**, Stokes I. The mechanics of the foot. In: Klennerman L The foot and its disorders. Oxford, England: Blackwell Scientific, 1991: 11-25
- 23 **Boulton A**. Peripheral neuropathy and the diabetic foot. *The Foot* 1992; **2**: 67-72 [DOI: 10.1016/0958-2592(92)90020-P]
- 24 **Frykberg RG**, Lavery LA, Pham H, Harvey C, Harkless L, Veves A. Role of neuropathy and high foot pressures in diabetic foot ulceration. *Diabetes Care* 1998; **21**: 1714-1719 [PMID: 9773736 DOI: 10.2337/diacare.21.10.1714]
- 25 **Bus SA**, Yang QX, Wang JH, Smith MB, Wunderlich R, Cavanagh PR. Intrinsic muscle atrophy and toe deformity in the diabetic neuropathic foot: a magnetic resonance imaging study. *Diabetes Care* 2002; **25**: 1444-1450 [PMID: 12145248 DOI: 10.2337/diacare.25.8.1444]
- 26 **Shaw JE**, Boulton AJ. The pathogenesis of diabetic foot problems: an overview. *Diabetes* 1997; **46** Suppl 2: S58-S61 [PMID: 9285501 DOI: 10.2337/diab.46.2.S58]
- 27 **Nabuurs-Franssen MH**, Houben AJ, Tooke JE, Schaper NC. The effect of polyneuropathy on foot microcirculation in Type II diabetes. *Diabetologia* 2002; **45**: 1164-1171 [PMID: 12189447 DOI: 10.1007/s00125-002-0872-z]
- 28 **England JD**, Gronseth GS, Franklin G, Miller RG, Asbury AK, Carter GT, Cohen JA, Fisher MA, Howard JF, Kinsella LJ, Latov N, Lewis RA, Low PA, Sumner AJ. Distal symmetrical polyneuropathy: a definition for clinical research. A report of the American Academy of Neurology, the American Association of Electrodiagnostic Medicine, and the American Academy of Physical Medicine and Rehabilitation. *Arch Phys Med Rehabil* 2005; **86**: 167-174 [PMID: 15641009 DOI: 10.1016/j.apmr.2004.09.011]
- 29 **Didangelos T**, Doupis J, Veves A. Painful diabetic neuropathy: clinical aspects. *Handb Clin Neurol* 2014; **126**: 53-61 [PMID: 25410214 DOI: 10.1016/B978-0-444-53480-4.00005-9]
- 30 **Ziegler D**, Mayer P, Gries FA. Evaluation of thermal, pain, and vibration sensation thresholds in newly diagnosed type 1 diabetic patients. *J Neurol Neurosurg Psychiatry* 1988; **51**: 1420-1424 [PMID: 3236020 DOI: 10.1136/jnnp.51.11.1420]
- 31 **Dyck PJ**. Detection, characterization, and staging of polyneuropathy: assessed in diabetics. *Muscle Nerve* 1988; **11**: 21-32 [PMID: 3277049 DOI: 10.1002/mus.880110106]
- 32 **Dyck PJ**, Sherman WR, Hallcher LM, Service FJ, O'Brien PC, Grina LA, Palumbo PJ, Swanson CJ. Human diabetic endoneurial sorbitol, fructose, and myo-inositol related to sural nerve morphometry. *Ann Neurol* 1980; **8**: 590-596 [PMID: 7212646 DOI: 10.1002/ana.410080608]
- 33 **Dyck PJ**, Karnes JL, Daube J, O'Brien P, Service FJ. Clinical and neuropathological criteria for the diagnosis and staging of diabetic polyneuropathy. *Brain* 1985; **108** (Pt 4): 861-880 [PMID: 4075076 DOI: 10.1093/brain/108.4.861]
- 34 **Dyck PJ**, Kratz KM, Lehman KA, Karnes JL, Melton LJ, O'Brien PC, Litchy WJ, Windebank AJ, Smith BE, Low PA. The Rochester Diabetic Neuropathy Study: design, criteria for types of neuropathy, selection bias, and reproducibility of neuropathic tests. *Neurology* 1991; **41**: 799-807 [PMID: 2046920 DOI: 10.1212/WNL.41.6.799]
- 35 **Dyck PJ**, Karnes J, O'Brien PC, Swanson CJ. Neuropathy Symptom Profile in health, motor neuron disease, diabetic neuropathy, and amyloidosis. *Neurology* 1986; **36**: 1300-1308 [PMID: 3762934 DOI: 10.1212/WNL.36.10.1300]
- 36 **Feldman EL**, Stevens MJ, Thomas PK, Brown MB, Canal N, Greene DA. A practical two-step quantitative clinical and electrophysiological assessment for the diagnosis and staging of diabetic neuropathy. *Diabetes Care* 1994; **17**: 1281-1289 [PMID: 7821168 DOI: 10.2337/diacare.17.11.1281]
- 37 **Veves A**, Manes C, Murray HJ, Young MJ, Boulton AJ. Painful neuropathy and foot ulceration in diabetic patients. *Diabetes Care* 1993; **16**: 1187-1189 [PMID: 8375249 DOI: 10.2337/diacare.16.8.1187]



- 38 **Meijer JW**, Smit AJ, Sonderen EV, Groothoff JW, Eisma WH, Links TP. Symptom scoring systems to diagnose distal polyneuropathy in diabetes: the Diabetic Neuropathy Symptom score. *Diabet Med* 2002; **19**: 962-965 [PMID: 12421436 DOI: 10.1046/j.1464-5491.2002.00819.x]
- 39 **Valk GD**, de Sonnaville JJ, van Houtum WH, Heine RJ, van Eijk JT, Bouter LM, Bertelsmann FW. The assessment of diabetic polyneuropathy in daily clinical practice: reproducibility and validity of Semmes Weinstein monofilaments examination and clinical neurological examination. *Muscle Nerve* 1997; **20**: 116-118 [PMID: 8995595 DOI: 10.1002/(SICI)1097-4598(199701)20:1<116::AID-MUS19>3.0.CO;2-2]
- 40 **Mayfield JA**, Sugarman JR. The use of the Semmes-Weinstein monofilament and other threshold tests for preventing foot ulceration and amputation in persons with diabetes. *J Fam Pract* 2000; **49**: S17-S29 [PMID: 11093555]
- 41 **Smieja M**, Hunt DL, Edelman D, Etchells E, Cornuz J, Simel DL. Clinical examination for the detection of protective sensation in the feet of diabetic patients. International Cooperative Group for Clinical Examination Research. *J Gen Intern Med* 1999; **14**: 418-424 [PMID: 10417599 DOI: 10.1046/j.1525-1497.1999.05208.x]
- 42 **Rith-Najarian SJ**, Stolusky T, Gohdes DM. Identifying diabetic patients at high risk for lower-extremity amputation in a primary health care setting. A prospective evaluation of simple screening criteria. *Diabetes Care* 1992; **15**: 1386-1389 [PMID: 1425105 DOI: 10.2337/diacare.15.10.1386]
- 43 **Pham H**, Armstrong DG, Harvey C, Harkless LB, Giurini JM, Veves A. Screening techniques to identify people at high risk for diabetic foot ulceration: a prospective multicenter trial. *Diabetes Care* 2000; **23**: 606-611 [PMID: 10834417 DOI: 10.2337/diacare.23.5.606]
- 44 **Liniger C**, Albeanu A, Bloise D, Assal JP. The tuning fork revisited. *Diabet Med* 1990; **7**: 859-864 [PMID: 2149682 DOI: 10.1111/j.1464-5491.1990.tb01319.x]
- 45 **Thivolet C**, el Farkh J, Petiot A, Simonet C, Tourniaire J. Measuring vibration sensations with graduated tuning fork. Simple and reliable means to detect diabetic patients at risk of neuropathic foot ulceration. *Diabetes Care* 1990; **13**: 1077-1080 [PMID: 2209306 DOI: 10.2337/diacare.13.10.1077]
- 46 **Boulton AJ**, Kubrusly DB, Bowker JH, Gadia MT, Quintero L, Becker DM, Skyler JS, Sosenko JM. Impaired vibratory perception and diabetic foot ulceration. *Diabet Med* 1986; **3**: 335-337 [PMID: 2949922 DOI: 10.1111/j.1464-5491.1986.tb00775.x]
- 47 **Young MJ**, Breddy JL, Veves A, Boulton AJ. The prediction of diabetic neuropathic foot ulceration using vibration perception thresholds. A prospective study. *Diabetes Care* 1994; **17**: 557-560 [PMID: 8082524 DOI: 10.2337/diacare.17.6.557]
- 48 **Arezzo JC**. The use of electrophysiology for the assessment of diabetic neuropathy. *Neurosci Res Comm* 1997; **21**: 13-23 [DOI: 10.1002/(SICI)1520-6769(199707)21:1<13::AID-NRC203>3.0.CO;2-P]
- 49 **Edwards JL**, Vincent AM, Cheng HT, Feldman EL. Diabetic neuropathy: mechanisms to management. *Pharmacol Ther* 2008; **120**: 1-34 [PMID: 18616962 DOI: 10.1016/j.pharmthera.2008.05.005]
- 50 **Gooch C**, Podwall D. The diabetic neuropathies. *Neurologist* 2004; **10**: 311-322 [PMID: 15518597 DOI: 10.1097/01.nrl.0000144733.61110.25]
- 51 **Thomas PK**. Nerve biopsy. *Diabet Med* 1997; **14**: 345-346 [PMID: 9171247 DOI: 10.1002/(SICI)1096-9136(199705)14:5<345::AID-DIA381>3.0.CO;2-B]
- 52 **Dahlin LB**, Eriksson KF, Sundkvist G. Persistent postoperative complaints after whole sural nerve biopsies in diabetic and non-diabetic subjects. *Diabet Med* 1997; **14**: 353-356 [PMID: 9171249 DOI: 10.1002/(SICI)1096-9136(199705)14:5<353::AID-DIA343>3.0.CO;2-E]
- 53 **Greene DA**, Arezzo JC, Brown MB. Effect of aldose reductase inhibition on nerve conduction and morphometry in diabetic neuropathy. Zenarestat Study Group. *Neurology* 1999; **53**: 580-591 [PMID: 10449124 DOI: 10.1212/WNL.53.3.580]
- 54 **Sima AA**, Calvani M, Mehra M, Amato A. Acetyl-L-carnitine improves pain, nerve regeneration, and vibratory perception in patients with chronic diabetic neuropathy: an analysis of two randomized placebo-controlled trials. *Diabetes Care* 2005; **28**: 89-94 [PMID: 15616239 DOI: 10.2337/diacare.28.1.89]
- 55 **McCarthy BG**, Hsieh ST, Stocks A, Hauer P, Macko C, Cornblath DR, Griffin JW, McArthur JC. Cutaneous innervation in sensory neuropathies: evaluation by skin biopsy. *Neurology* 1995; **45**: 1848-1855 [PMID: 7477980]
- 56 **Holland NR**, Crawford TO, Hauer P, Cornblath DR, Griffin JW, McArthur JC. Small-fiber sensory neuropathies: clinical course and neuropathology of idiopathic cases. *Ann Neurol* 1998; **44**: 47-59 [PMID: 9667592 DOI: 10.1002/ana.410440111]
- 57 **Polydefkis M**, Griffin JW, McArthur J. New insights into diabetic polyneuropathy. *JAMA* 2003; **290**: 1371-1376 [PMID: 12966130 DOI: 10.1001/jama.290.10.1371]
- 58 **Akbari CM**, Macsata R, Smith BM, Sidawy AN. Overview of the diabetic foot. *Semin Vasc Surg* 2003; **16**: 3-11 [PMID: 12644970 DOI: 10.1053/svas.2003.50001]
- 59 **Al-Delaimy WK**, Merchant AT, Rimm EB, Willett WC, Stampfer MJ, Hu FB. Effect of type 2 diabetes and its duration on the risk of peripheral arterial disease among men. *Am J Med* 2004; **116**: 236-240 [PMID: 14969651 DOI: 10.1016/j.amjmed.2003.09.038]
- 60 **Murabito JM**, D'Agostino RB, Silbershatz H, Wilson WF. Intermittent claudication. A risk profile from The Framingham Heart Study. *Circulation* 1997; **96**: 44-49 [PMID: 9236415 DOI: 10.1161/01.CIR.96.1.44]
- 61 **McDermott MM**, Greenland P, Liu K, Guralnik JM, Criqui MH, Dolan NC, Chan C, Celic L, Pearce WH, Schneider JR, Sharma L, Clark E, Gibson D, Martin GJ. Leg symptoms in peripheral arterial disease: associated clinical characteristics and functional impairment. *JAMA* 2001; **286**: 1599-1606 [PMID: 11585483 DOI: 10.1001/jama.286.13.1599]
- 62 **American Diabetes Association**. Peripheral arterial disease in people with diabetes. *Diabetes Care* 2003; **26**: 3333-3341 [PMID: 14633825 DOI: 10.2337/diacare.26.12.3333]
- 63 **Hirsch AT**, Haskal ZJ, Hertzner NR, Bakal CW, Creager MA, Halperin JL, Hiratzka LF, Murphy WR, Olin JW, Puschett JB, Rosenfield KA, Sacks D, Stanley JC, Taylor LM, Jr., White CJ, White J, White RA, Antman EM, Smith SC, Jr., Adams CD, Anderson JL, Faxon DP, Fuster V, Gibbons RJ, Halperin JL, Hiratzka LF, Hunt SA, Jacobs AK, Nishimura R, Ornato JP, Page RL, Riegel B, American Association for Vascular S, Society for Vascular S, Society for Cardiovascular A, Interventions, Society for Vascular M, Biology, Society of Interventional R, Guidelines AATFoP, American Association of C, Pulmonary R, National Heart L, Blood I, Society for Vascular N, TransAtlantic Inter-Society C, Vascular Disease F. Acc/aha 2005 guidelines for the management of patients with peripheral arterial disease (lower extremity, renal, mesenteric, and abdominal aortic): Executive summary a collaborative report from the american association for vascular surgery/society for vascular surgery, society for cardiovascular angiography and interventions, society for vascular medicine and biology, society of interventional radiology, and the acc/aha task force on practice guidelines (writing committee to develop guidelines for the management of patients with peripheral arterial disease) endorsed by the american association of cardiovascular and pulmonary rehabilitation; national heart, lung, and blood institute; society for vascular nursing; transatlantic inter-society consensus; and vascular disease foundation. *J Am Coll Cardiol* 2006; **47**: 1239-1312 [PMID: 16545667 DOI: 10.1016/j.jacc.2005.10.009]
- 64 **Halperin JL**. Evaluation of patients with peripheral vascular disease. *Thromb Res* 2002; **106**: V303-V311 [PMID: 12359343 DOI: 10.1016/S0049-3848(01)00366-8]
- 65 **Brooks B**, Dean R, Patel S, Wu B, Molyneaux L, Yue DK. TBI or not TBI: that is the question. Is it better to measure toe pressure than ankle pressure in diabetic patients? *Diabet Med* 2001; **18**: 528-532 [PMID: 11553180 DOI: 10.1046/j.1464-5491.2001.00493.x]
- 66 **Rutherford RB**, Lowenstein DH, Klein MF. Combining segmental

- systolic pressures and plethysmography to diagnose arterial occlusive disease of the legs. *Am J Surg* 1979; **138**: 211-218 [PMID: 380378 DOI: 10.1016/0002-9610(79)90372-6]
- 67 **Strandness Jr DE**. Peripheral arterial system. In: Strandness Jr DE Duplex scanning in vascular disorders. Philadelphia, PA: Lippincott Williams & Wilkins, 2002: 118-143 [DOI: 10.1385/1-59259-331-3: 55]
  - 68 **Whelan JF**, Barry MH, Moir JD. Color flow Doppler ultrasonography: comparison with peripheral arteriography for the investigation of peripheral vascular disease. *J Clin Ultrasound* 1992; **20**: 369-374 [PMID: 1328307 DOI: 10.1002/jcu.1870200602]
  - 69 **Allard L**, Cloutier G, Durand LG, Roederer GO, Langlois YE. Limitations of ultrasonic duplex scanning for diagnosing lower limb arterial stenoses in the presence of adjacent segment disease. *J Vasc Surg* 1994; **19**: 650-657 [PMID: 8164280 DOI: 10.1016/S0741-5214(94)70038-9]
  - 70 **Larch E**, Minar E, Ahmadi R, Schnürer G, Schneider B, Stümpflen A, Ehringer H. Value of color duplex sonography for evaluation of tibioperoneal arteries in patients with femoropopliteal obstruction: a prospective comparison with anterograde intraarterial digital subtraction angiography. *J Vasc Surg* 1997; **25**: 629-636 [PMID: 9129617 DOI: 10.1016/S0741-5214(97)70288-X]
  - 71 **Carpenter JP**, Baum RA, Holland GA, Barker CF. Peripheral vascular surgery with magnetic resonance angiography as the sole preoperative imaging modality. *J Vasc Surg* 1994; **20**: 861-869; discussion 869-871 [PMID: 7990180 DOI: 10.1016/0741-5214(94) 90222-4]
  - 72 **Leiner T**, Kessels AG, Nelemans PJ, Vasbinder GB, de Haan MW, Kitslaar PE, Ho KY, Tordoir JH, van Engelshoven JM. Peripheral arterial disease: comparison of color duplex US and contrast-enhanced MR angiography for diagnosis. *Radiology* 2005; **235**: 699-708 [PMID: 15858107 DOI: 10.1148/radiol.2352040089]
  - 73 **Oyibo SO**, Jude EB, Tarawneh I, Nguyen HC, Harkless LB, Boulton AJ. A comparison of two diabetic foot ulcer classification systems: the Wagner and the University of Texas wound classification systems. *Diabetes Care* 2001; **24**: 84-88 [PMID: 11194247 DOI: 10.2337/diacare.24.1.84]
  - 74 **Treece KA**, Macfarlane RM, Pound N, Game FL, Jeffcoate WJ. Validation of a system of foot ulcer classification in diabetes mellitus. *Diabet Med* 2004; **21**: 987-991 [PMID: 15317603 DOI: 10.1111/j.1464-5491.2004.01275.x]
  - 75 **Schaper NC**. Diabetic foot ulcer classification system for research purposes: a progress report on criteria for including patients in research studies. *Diabetes Metab Res Rev* 2004; **20** Suppl 1: S90-S95 [PMID: 15150820 DOI: 10.1002/dmrr.464]
  - 76 **Lipsky BA**, Berendt AR, Deery HG, Embil JM, Joseph WS, Karchmer AW, LeFrock JL, Lew DP, Mader JT, Norden C, Tan JS. Diagnosis and treatment of diabetic foot infections. *Clin Infect Dis* 2004; **39**: 885-910 [PMID: 15472838 DOI: 10.1086/424846]
  - 77 **Falabella AF**. Debridement and wound bed preparation. *Dermatol Ther* 2006; **19**: 317-325 [PMID: 17199674 DOI: 10.1111/j.1529-8019.2006.00090.x]
  - 78 **Veves A**, Giurini J, LoGerfo F. The diabetic foot: Medical and surgical management. Totowa, NJ: Humana Press, 2006 [DOI: 10.1007/978-1-59745-075-1]
  - 79 **Steed DL**, Donohoe D, Webster MW, Lindsley L. Effect of extensive debridement and treatment on the healing of diabetic foot ulcers. Diabetic Ulcer Study Group. *J Am Coll Surg* 1996; **183**: 61-64 [PMID: 8673309]
  - 80 **Bale S**. A guide to wound debridement. *J Wound Care* 1997; **6**: 179-182 [PMID: 9256718]
  - 81 **Hultén L**. Dressings for surgical wounds. *Am J Surg* 1994; **167**: 42S-44S; discussion 44S-45S [PMID: 8109684]
  - 82 **Sherman RA**. Maggot therapy for treating diabetic foot ulcers unresponsive to conventional therapy. *Diabetes Care* 2003; **26**: 446-451 [PMID: 12547878 DOI: 10.2337/diacare.26.2.446]
  - 83 **Mumcuoglu KY**, Ingber A, Gilead L, Stessman J, Friedmann R, Schulman H, Bichucher H, Ioffe-Uspensky I, Miller J, Galun R, Raz I. Maggot therapy for the treatment of intractable wounds. *Int J Dermatol* 1999; **38**: 623-627 [PMID: 10487456 DOI: 10.1046/j.1365-4362.1999.00770.x]
  - 84 **Sherman RA**, Wyle F, Vulpe M. Maggot therapy for treating pressure ulcers in spinal cord injury patients. *J Spinal Cord Med* 1995; **18**: 71-74 [PMID: 7640976]
  - 85 **Doupis J**, Veves A. Classification, diagnosis, and treatment of diabetic foot ulcers. *Wounds* 2008; **20**: 117-126 [PMID: 25942412]
  - 86 **Nabuurs-Franssen MH**, Slegers R, Huijberts MS, Wijnen W, Sanders AP, Walenkamp G, Schaper NC. Total contact casting of the diabetic foot in daily practice: a prospective follow-up study. *Diabetes Care* 2005; **28**: 243-247 [PMID: 15677773 DOI: 10.2337/diacare.28.2.243]
  - 87 **Lavery LA**, Vela SA, Lavery DC, Quebedeaux TL. Reducing dynamic foot pressures in high-risk diabetic subjects with foot ulcerations. A comparison of treatments. *Diabetes Care* 1996; **19**: 818-821 [PMID: 8842597 DOI: 10.2337/diacare.19.8.818]
  - 88 **Birke J**, Fred B, Krieger L, Sliman K. The effectiveness of an accommodative dressing in offloading pressure over areas of previous metatarsal head ulceration. *Wounds* 2003; **15**: 33-39
  - 89 **Younes NA**, Bakri FG. Diabetic foot infection. *Saudi Med J* 2006; **27**: 596-603 [PMID: 16680244]
  - 90 **Lipsky BA**, Pecoraro RE, Wheat LJ. The diabetic foot. Soft tissue and bone infection. *Infect Dis Clin North Am* 1990; **4**: 409-432 [PMID: 2212597]
  - 91 **Sumpio BE**, Aruny J, Blume PA. The multidisciplinary approach to limb salvage. *Acta Chir Belg* 2004; **104**: 647-653 [PMID: 15663269]
  - 92 **Queen D**, Orsted H, Sanada H, Sussman G. A dressing history. *Int Wound J* 2004; **1**: 59-77 [PMID: 16722898 DOI: 10.1111/j.1742-4801.2004.0009.x]
  - 93 **Sibbald RG**, Torrance G, Hux M, Attard C, Milkovich N. Cost-effectiveness of becaplermin for nonhealing neuropathic diabetic foot ulcers. *Ostomy Wound Manage* 2003; **49**: 76-84 [PMID: 14652415]
  - 94 **Moura LI**, Dias AM, Carvalho E, de Sousa HC. Recent advances on the development of wound dressings for diabetic foot ulcer treatment--a review. *Acta Biomater* 2013; **9**: 7093-7114 [PMID: 23542233 DOI: 10.1016/j.actbio.2013.03.033]
  - 95 **Bryant RA**. Acute and chronic wounds. St. Louis, MO: Mosby, 2000
  - 96 **McDonald A**, Lesage P. Palliative management of pressure ulcers and malignant wounds in patients with advanced illness. *J Palliat Med* 2006; **9**: 285-295 [PMID: 16629558 DOI: 10.1089/jpm.2006.9.285]
  - 97 **Kalinski C**, Schnepf M, Laboy D, Hernandez L, Nusbaum J, McGrinder B, Comfort C, Alvarez OM. Effectiveness of a topical formulation containing metronidazole for wound odor and exudate control. *Wounds* 2005; **17**: 84-90
  - 98 **Newman V**, Allwood M, Oakes R. The use of metronidazole gel to control the smell of malodorous lesions. *Palliat Med* 1989; **3**: 303-305 [DOI: 10.1177/026921638900300412]
  - 99 **Viswanathan V**, Kesavan R, Kavitha KV, Kumpatla S. A pilot study on the effects of a polyherbal formulation cream on diabetic foot ulcers. *Indian J Med Res* 2011; **134**: 168-173 [PMID: 21911968]
  - 100 **Geronemus RG**, Mertz PM, Eaglstein WH. Wound healing. The effects of topical antimicrobial agents. *Arch Dermatol* 1979; **115**: 1311-1314 [PMID: 507884 DOI: 10.1001/archderm.1979.040101100 17016]
  - 101 **Kashyap A**, Beezhod D, Wiseman J, Beck WC. Effect of povidone iodine dermatologic ointment on wound healing. *Am Surg* 1995; **61**: 486-491 [PMID: 7762895]
  - 102 **Innes ME**, Umraw N, Fish JS, Gomez M, Cartotto RC. The use of silver coated dressings on donor site wounds: a prospective, controlled matched pair study. *Burns* 2001; **27**: 621-627 [PMID: 11525858 DOI: 10.1016/S0305-4179(01)00015-8]
  - 103 **Kilinc H**, Sensöz O, Ozdemir R, Unlü RE, Baran C. Which dressing for split-thickness skin graft donor sites? *Ann Plast Surg* 2001; **46**: 409-414 [PMID: 11324884 DOI: 10.1097/0000637-200 104000-00010]
  - 104 **Field FK**, Kerstein MD. Overview of wound healing in a moist environment. *Am J Surg* 1994; **167**: 2S-6S [PMID: 8109679 DOI: 10.1046/j.1365-4362.1999.00770.x]

- 10.1016/0002-9610(94)90002-7]
- 105 **Schultz GS**, Sibbald RG, Falanga V, Ayello EA, Dowsett C, Harding K, Romanelli M, Stacey MC, Teot L, Vanscheidt W. Wound bed preparation: a systematic approach to wound management. *Wound Repair Regen* 2003; **11** Suppl 1: S1-28 [PMID: 12654015 DOI: 10.1046/j.1524-475X.11.s2.1.x]
- 106 **Trudgian J**. Investigating the use of Aquaform Hydrogel in wound management. *Br J Nurs* 2000; **9**: 943-948 [PMID: 11261031 DOI: 10.12968/bjon.2000.9.14.943]
- 107 **Mulder GD**. Cost-effective managed care: gel versus wet-to-dry for debridement. *Ostomy Wound Manage* 1995; **41**: 68-70, 72, 74 passim [PMID: 7598779]
- 108 **Kavitha KV**, Tiwari S, Purandare VB, Khedkar S, Bhosale SS, Unnikrishnan AG. Choice of wound care in diabetic foot ulcer: A practical approach. *World J Diabetes* 2014; **5**: 546-556 [PMID: 25126400 DOI: 10.4239/wjd.v5.i4.546]
- 109 **Lohmann M**, Thomsen JK, Edmonds ME, Harding KG, Apelqvist J, Gottrup F. Safety and performance of a new non-adhesive foam dressing for the treatment of diabetic foot ulcers. *J Wound Care* 2004; **13**: 118-120 [PMID: 15045807 DOI: 10.12968/jowc.2004.13.3.26591]
- 110 **Cowan T**. Wound care handbook. London: Mark Allen Healthcare, 2011
- 111 **Molan PC**, Betts JA. Using honey to heal diabetic foot ulcers. *Adv Skin Wound Care* 2008; **21**: 313-316 [PMID: 18600072 DOI: 10.1097/01.ASW.0000323523.38800.9f]
- 112 **Molan PC**. The evidence supporting the use of honey as a wound dressing. *Int J Low Extrem Wounds* 2006; **5**: 40-54 [PMID: 16543212 DOI: 10.1177/1534734605286014]
- 113 **Fitton AR**, Drew P, Dickson WA. The use of a bilaminate artificial skin substitute (Integra) in acute resurfacing of burns: an early experience. *Br J Plast Surg* 2001; **54**: 208-212 [PMID: 11254411 DOI: 10.1054/bjps.2000.3525]
- 114 **Veerapur BG**, Uppin I, Babu YR. Chronic diabetic wound healing: Recombinant pdgf v/s normal saline. *J Pharmaceut Biomed* 2012; **24**: 118-120
- 115 **Fernández-Montequín JI**, Valenzuela-Silva CM, Díaz OG, Savigne W, Sancho-Soutelo N, Rivero-Fernández F, Sánchez-Penton P, Morejón-Vega L, Artaza-Sanz H, García-Herrera A, González-Benavides C, Hernández-Cañete CM, Vázquez-Proenza A, Berlanga-Acosta J, López-Saura PA. Intra-lesional injections of recombinant human epidermal growth factor promote granulation and healing in advanced diabetic foot ulcers: multicenter, randomised, placebo-controlled, double-blind study. *Int Wound J* 2009; **6**: 432-443 [PMID: 20051095 DOI: 10.1111/j.1742-481X.2009.00641.x]
- 116 **Vijaykumar H**, Pai SA, Pandey V, Kamble P. Comparative study of collagenase and papain-urea based preparations in the management of chronic nonhealing limb ulcers. *Indian J Sci Technol* 2011; **4**: 1096-1100 [DOI: 10.17485/ijst/2011/v4i9/30237]
- 117 **Andros G**, Armstrong DG, Attinger CE, Boulton AJ, Frykberg RG, Joseph WS, Lavery LA, Morbach S, Niezgoda JA, Toursarkissian B. Consensus statement on negative pressure wound therapy (V.A.C. Therapy) for the management of diabetic foot wounds. *Ostomy Wound Manage* 2006; **Suppl**: 1-32 [PMID: 17007488]
- 118 **Venturi ML**, Attinger CE, Mesbahi AN, Hess CL, Graw KS. Mechanisms and clinical applications of the vacuum-assisted closure (VAC) Device: a review. *Am J Clin Dermatol* 2005; **6**: 185-194 [PMID: 15943495 DOI: 10.2165/00128071-200506030-00005]
- 119 **Petrie N**, Potter M, Banwell P. The management of lower extremity wounds using topical negative pressure. *Int J Low Extrem Wounds* 2003; **2**: 198-206 [PMID: 15866848 DOI: 10.1177/1534734603261067]
- 120 **Kot J**, Desola J, Simao AG, Gough-Allen R, Houman R, Meliet JL, Galland F, Mortensen C, Mueller PH, Sippinen S. A European code of good practice for hyperbaric oxygen therapy. *Int Marit Health* 2004; **55**: 121-130 [PMID: 15881548]
- 121 **Strauss MB**. Hyperbaric oxygen as an intervention for managing wound hypoxia: its role and usefulness in diabetic foot wounds. *Foot Ankle Int* 2005; **26**: 15-18 [PMID: 15680113]
- 122 **Mathieu D**. Role of hyperbaric oxygen therapy in the management of lower extremity wounds. *Int J Low Extrem Wounds* 2006; **5**: 233-235 [PMID: 17088598 DOI: 10.1177/1534734606294450]
- 123 **Kessler L**, Bilbault P, Ortéga F, Grasso C, Passemard R, Stephan D, Pinget M, Schneider F. Hyperbaric oxygenation accelerates the healing rate of nonischemic chronic diabetic foot ulcers: a prospective randomized study. *Diabetes Care* 2003; **26**: 2378-2382 [PMID: 12882865 DOI: 10.2337/diacare.26.8.2378]
- 124 **Fernandes TD**. Hyperbaric medicine. *Acta Med Port* 2009; **22**: 323-334 [PMID: 19909659]
- 125 **Shah J**. Hyperbaric oxygen therapy. *J Am Col Certif Wound Spec* 2010; **2**: 9-13 [PMID: 24527137 DOI: 10.1016/j.jcws.2010.04.001]
- 126 **Goulon M**, Bakker D, Marroni A, Mathieu D, Wattel F, Bitterman N. 7th European Consensus Conference on Hyperbaric Medicine. 2004. Available from: URL: [http://www.echm.org/documents/ECHM\\_7th\\_Consensus\\_Conference\\_Lille\\_2004.pdf](http://www.echm.org/documents/ECHM_7th_Consensus_Conference_Lille_2004.pdf)
- 127 **Hart CA**, Tsui J, Khanna A, Abraham DJ, Baker DM. *Exp Biol Med* (Maywood) 2013; **238**: 1118-1126 [DOI: 10.1177/1535370213503275]

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Editorial Board Member of *World Journal of Diabetes*, José M Gómez-Sáez, MD, PhD, Research Fellow, Endocrinology Service, Hospital Universitario de Bellvitg, 08907 Barcelona, Spain

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*World Journal of Diabetes* (*World J Diabetes*, *WJD*, online ISSN 1948-9358, DOI: 10.4239), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJD* covers topics concerning  $\alpha$ ,  $\beta$ ,  $\delta$  and PP cells of the pancreatic islet, the effect of insulin and insulinresistance, pancreatic islet transplantation, adipose cells and obesity.

We encourage authors to submit their manuscripts to *WJD*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great clinical significance.

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*World Journal of Diabetes*

Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China  
Telephone: +86-10-85381891

Fax: +86-10-85381893

E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)

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Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

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## Diabetes epidemic sweeping the Arab world

Bisher Abuyassin, Ismail Laher

Bisher Abuyassin, Ismail Laher, Department of Pharmacology and Therapeutics, Faculty of Medicine, University of British Columbia, Vancouver, BC V6T 1Z3, Canada

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**Correspondence to:** Dr. Ismail Laher, PhD, Department of Pharmacology and Therapeutics, Faculty of Medicine, University of British Columbia, 2176 Health Science Mall, Medical Block C, Vancouver, BC V6T 1Z3, Canada. [ilaher@mail.ubc.ca](mailto:ilaher@mail.ubc.ca)  
 Telephone: +1-604-8225882  
 Fax: +1-604-8226012

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### Abstract

The prevalence of type-2 diabetes mellitus (T2DM) has increased dramatically during the last 2 decades, a fact driven by the increased prevalence of obesity, the primary risk factor for T2DM. The figures for diabetes in the Arab world are particularly startling as the number of people with diabetes is projected to increase by 96.2%

by 2035. Genetic risk factors may play a crucial role in this uncontrolled rise in the prevalence of T2DM in the Middle Eastern region. However, factors such as obesity, rapid urbanization and lack of exercise are other key determinants of this rapid increase in the rate of T2DM in the Arab world. The unavailability of an effective program to defeat T2DM has serious consequences on the increasing rise of this disease, where available data indicates an unusually high prevalence of T2DM in Arabian children less than 18 years old. Living with T2DM is problematic as well, since T2DM has become the 5<sup>th</sup> leading cause of disability, which was ranked 10<sup>th</sup> as recently as 1990. Giving the current status of T2DM in the Arab world, a collaborative international effort is needed for fighting further spread of this disease.

**Key words:** Diabetes; Arab world; Epidemiology; Etiology; Risk factors and complication

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**Core tip:** The Middle Eastern and North African region has the second highest rate of increases in diabetes anywhere in the world. We comprehensively review type-2 diabetes mellitus (T2DM) in adults and children from 22 Arab speaking countries by reviewing data published from 1980 to 2015; this allowed us to have a better view of the trends in the dramatic increases of T2DM impacting the Arabic region. We also discuss the etiology of this uncontrolled medical crisis and the most commonly reported complications in these Arab speaking countries. Finally, we highlight a number of crucial data that appear to be unavailable but which may be essential for a more comprehensive understanding of the diabetes epidemic sweeping the Arabian region.

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## INTRODUCTION

Diabetes mellitus (DM) affects more than 382 million people around the world, of whom 90% are diagnosed with type-2 DM (T2DM)<sup>[1]</sup>. The prevalence of T2DM has increased dramatically during the last 2 decades<sup>[1]</sup>. The Arab world is not immune from this epidemic in the prevalence of T2DM. In fact, the Middle Eastern and North African region has the second highest rate of increase in diabetes globally, with the number of people with diabetes is projected to increase by 96.2% in 2035<sup>[1]</sup>. Having T2DM increases the burden for both patients and their caregivers, and of particular concern for Arab governments is the large economic burden of diabetes in terms of cost of treatment, management of complications, disability and loss of productivity<sup>[2-4]</sup>. The factors associated with T2DM seem more pronounced in the Arab world. Although genetic risk factors can't be ruled out in the context of T2DM in the Arab world, factors such as obesity, rapid urbanization and lack of exercise are key determinants of the rapid increase of the rate of T2DM among the Arab world<sup>[5]</sup>.

The unavailability of an effective program to contain the dramatic rise in T2DM in the Arab world has led to a serious development in the disease's course, where current data indicates that T2DM can't be ruled out in children less than 18 years old. Children diagnosed with T2DM come from all countries in the Arab world and obesity is a hallmark feature in this age group<sup>[6,7]</sup>.

This review will initially address the epidemiology of T2DM in the Arab world, followed by the etiology of the disease, complication and risk factors of T2DM, and finally will discuss some suggestions related to research on T2DM in the Arab world.

## RESEARCH METHODS

We undertook a search of the medical literature using the PubMed, EMBASE and Ovid databases for articles published in English language between 1980 and 2015, and included the following keywords: Diabetes, Arab world, epidemiology, etiology, risk factors and complication, or their corresponding MeSH term synonyms. Among 22 Arabic countries, a total of 2347 papers were identified and screened by title and/or abstract (Figure 1). To ensure that we included the highest number of epidemiological studies from each country, we did not set any limitations on the study design in our exclusion criteria. However, animal or genetic studies, studies not relevant to T2DM, studies on the effects of treatment, and non-primary data such as review articles or adherence studies were excluded (Figure 2). A total of 85 studies were added in the article and were reviewed in full. Among these, 3 studies were concerning Arab immigrants in different countries not listed in Figure 1, and 1 study (not PubMed indexed) was obtained from the references of other paper.

## EPIDEMIOLOGY

### Adults

Based on the International Diabetes Federation (IDF) estimates from 2013<sup>[1]</sup>, three countries from the Arabic world are among the top 10 countries worldwide for the prevalence of T2DM; these countries are Saudi Arabia, Kuwait and Qatar. The data obtained from each country in the Arabic world reports variation regarding prevalence of T2DM. However, differences in reported prevalence of T2DM within each country can be attributed to the study design, population and diagnostic methods used to obtain these data. The data clearly confirms that the prevalence of T2DM has increased dramatically during the past two decades. For example, studies from Saudi Arabia in the 80's indicated that prevalence of T2DM was between 2.4%-4.3%<sup>[8,9]</sup>, while recent a study in Saudi Arabia indicated a dramatic increase in the rate of T2DM with an estimated prevalence of 25.4%<sup>[10]</sup>. This pattern of massive increases in the rate of T2DM is similar for Iraq, Oman and other countries within the Arabic world (Table 1).

### Children and adolescents

Due to the recent recognition of T2DM during childhood, limited data are available worldwide. This unusual age-related disease has become a focus of attention for medical organizations around the world. A recent analysis showed a significant increase of 30.5% in the prevalence of T2DM among children and adolescents aged 10-19 years old in United States from 2001 to 2009<sup>[11]</sup>. The international society for pediatric and adolescents diabetes has published comprehensive guidelines for screening, diagnosis and treatment of T2DM in children and adolescents<sup>[12]</sup>.

Not surprisingly, data from the Arab world show similar figures for childhood T2DM. Perhaps more worrying is that recent data from Saudi Arabia reports an age-specific prevalence of 1 per 1000 for T2DM in children less than 18 years old<sup>[7]</sup>, which was similar to the highest prevalence found in specific groups (American Indian and African American) in United States<sup>[12]</sup>. Additional findings on childhood T2DM are summarized in Table 2.

## RISK FACTORS

A number of risk factors could account for the uncontrolled rise in T2DM in the Arab world, with genetic factors likely to play an important role. However, a number of modifiable risk factors such as obesity, rapid urbanization and its associated changes in dietary habits and lack of physical activity are also important determinants in the etiology of T2DM. Other considerations such as multiple pregnancies and a lack of health education may be unique challenges in the diabetes epidemic in the Arab world.

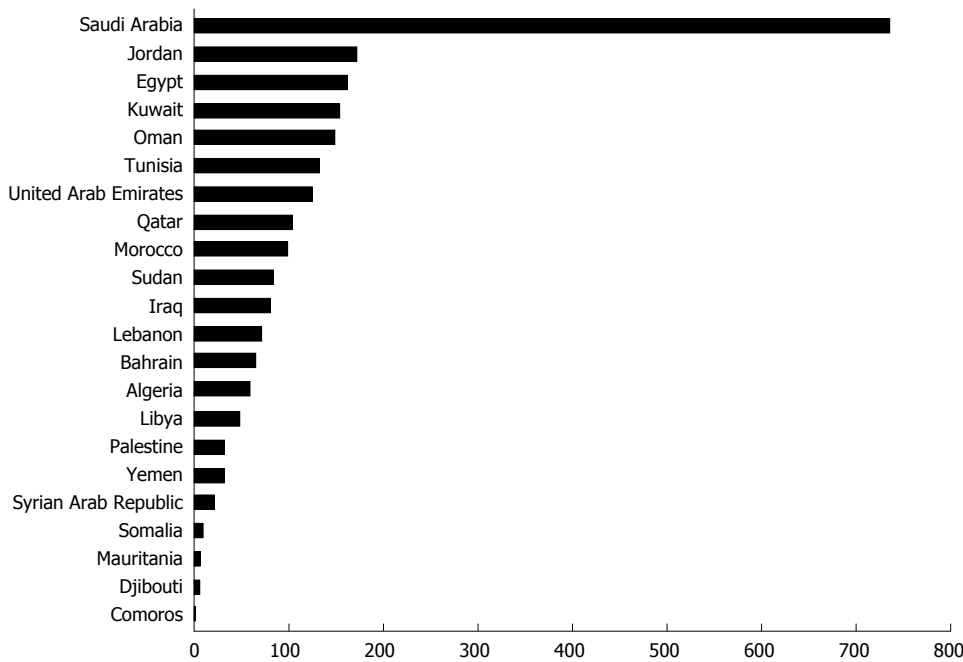


Figure 1 Number of studies reviewed by title and/or abstract for each Arabic country.

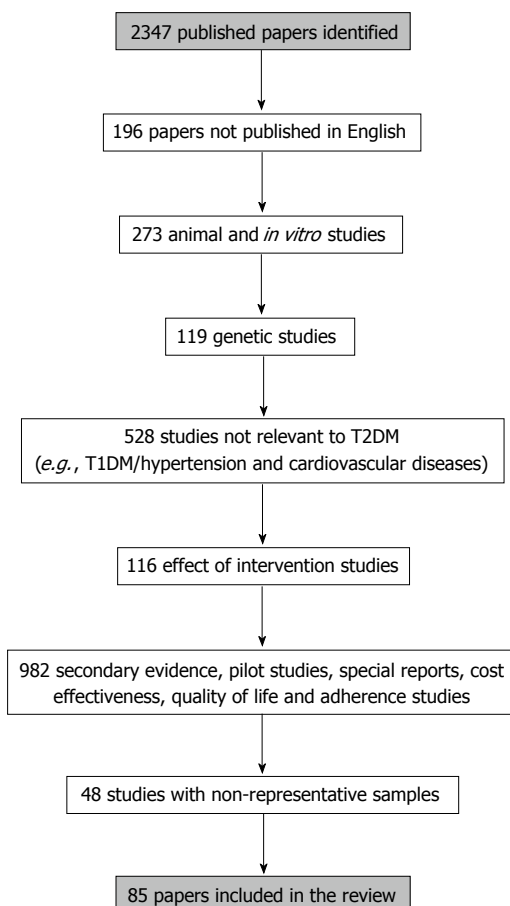


Figure 2 Research scheme and exclusion criteria. T2DM: Type-2 diabetes mellitus; T1DM: Type-1 diabetes mellitus.

### Obesity

Obesity represent a large component in the pathogenesis of T2DM<sup>[13]</sup>, and is by far the primary risk factor for the

increased prevalence of T2DM globally<sup>[14]</sup>. Obesity not only worsens the prognosis of T2DM, but obese diabetics suffer higher rates of microvascular complications and mortality<sup>[15-18]</sup>. Obesity-related T2DM has been investigated in several countries in the Arab world. In Saudi Arabia, a study of 14252 diabetic patients reported that more than half of the obese diabetics had poor glycemic control<sup>[19]</sup>. Additionally, the first national health survey in Kuwait indicates that 48% obese males and 77% obese females were also diabetics<sup>[20]</sup>, confirming a significant association between obesity and diabetes [odds ratio (OR) of 2.87] in the Kuwaiti population. In Oman and Qatar, 60.1% and 59.7% of the diabetic patients were obese respectively<sup>[21,22]</sup>. The average body mass index (BMI) in Palestinian and Lebanese diabetics was 33.7 kg/m<sup>2</sup> and 30.8 kg/m<sup>2</sup> respectively<sup>[23,24]</sup>. Nevertheless, obesity is significantly associated with diabetes even in some Arab countries such as Sudan and Tunisia where the prevalence of diabetes can be described as relatively low - the relative risk of developing T2DM was 1.74 (95%CI: 1.32-2.28) in obese Sudanese subjects and the OR of having T2DM was 1.61 (95%CI: 1.34-1.93) in obese Tunisians<sup>[25,26]</sup>.

A recent analysis of 46 Muslim countries (where Muslims represent > 50% of the population) estimated the prevalence of overweight subjects reported that in the Eastern Mediterranean region, females were more likely to be overweight than males (42.1% vs 33%)<sup>[27]</sup>. Interestingly, those living in Arab countries were 2.92 (95%CI: 2.86-2.97) times more likely to be overweight compared to those living in non-Arab Muslim countries.

### Rapid urbanization

Urbanizing of many rural areas within the Arab world carries with it many advantages in term of access to



**Table 1** Prevalence of type-2 diabetes mellitus in adult Arab population

Ref.	Country	Year	Sample size	Main findings	Diagnostic technique
Bacchus <i>et al</i> <sup>[8]</sup>	Saudi Arabia	1982	1385	Prevalence of diabetes: 2.4%	WHO criteria
Fatani <i>et al</i> <sup>[9]</sup>	Saudi Arabia	1987	5222	Prevalence of diabetes: 4.3%	Random capillary blood glucose
Al-Nuaim <i>et al</i> <sup>[28]</sup>	Saudi Arabia	1997	13177	Prevalence of diabetes in urban males and females: 12%, 14% Prevalence of diabetes in rural males and females: 7%, 7.7%	Random plasma glucose
Rahman Al-Nuaim <i>et al</i> <sup>[47]</sup>	Saudi Arabia	1997	2059	Prevalence of T2DM in obese males: 26.0% Prevalence of T2DM in non-obese males: 8.6% ( $P < 0.001$ ) Prevalence of T2DM in obese females: 23.5% Prevalence of T2DM in non-obese females: 4.4% ( $P < 0.0001$ )	OGTT
el-Hazmi <i>et al</i> <sup>[48]</sup>	Saudi Arabia	2000	14660	Prevalence of obesity in males: 13.05% Prevalence of obesity in females: 20.26% Prevalence of obesity in diabetics: 29.98% Prevalence of obesity in non-diabetics: 15.87% ( $P < 0.0001$ )	FBG
Al-Nozha <i>et al</i> <sup>[49]</sup>	Saudi Arabia	2004	16917	Prevalence of diabetes: 23.7%	FPG
Almajwal <i>et al</i> <sup>[50]</sup>	Saudi Arabia	2009	195851	Prevalence of diabetes: 17.2%	FBG
Al-Rubeaan <i>et al</i> <sup>[10]</sup>	Saudi Arabia	2014	18034	Prevalence of diabetes: 25.4%	FPG
Al-Rubeaan <i>et al</i> <sup>[51]</sup>	Saudi Arabia	2014	53370	Prevalence of abnormal glucose metabolism: 34.5%	FPG
El Bcheraoui <i>et al</i> <sup>[52]</sup>	Saudi Arabia	2014	10735	Prevalence of diabetes: 13.4%	FPG
Abdella <i>et al</i> <sup>[53]</sup>	Kuwait	1996	8336	Prevalence of diabetes: 7.6%	Medical chart review
Al Khalaf <i>et al</i> <sup>[54]</sup>	Kuwait	2010	560	Prevalence of diabetes: 21.4%	FBG
Alarouj <i>et al</i> <sup>[20]</sup>	Kuwait	2013	1970	Prevalence of diabetes: 17.9%	FPG
Al Zurba <i>et al</i> <sup>[55]</sup>	Bahrain	1996	498	Prevalence of diabetes: 25.5%	FPG
Malik <i>et al</i> <sup>[56]</sup>	UAE	2005	5758	Prevalence of T2DM: 20.2%	FPG
Saadi <i>et al</i> <sup>[57]</sup>	UAE	2007	2455	Prevalence of diagnosed T2DM: 10.5% Prevalence of undiagnosed T2DM: 6.6%	FBG
Mansour <i>et al</i> <sup>[58]</sup>	Iraq	2007	13730	Incidence of T2DM: 6.8%	FPG
Mansour <i>et al</i> <sup>[59]</sup>	Iraq	2014	5445	Prevalence of T2DM: 19.7%	FPG
Al-Moosa <i>et al</i> <sup>[21]</sup>	Oman	2006	5840	Prevalence of T2DM: 11.6%	FPG
Al-Lawati <i>et al</i> <sup>[60]</sup>	Oman	2015	NA	Age-adjusted prevalence of T2DM: 10.4% to 21.1%	FPG and OGTT
Musaiger <i>et al</i> <sup>[61]</sup>	Qatar	2005	535	Prevalence of T2DM among obese females $\geq 50$ yr: 51.4%	Self-reported diabetes
Bener <i>et al</i> <sup>[22]</sup>	Qatar	2009	1117	Prevalence of T2DM: 16.7%	FBG and OGTT
Al-Habori <i>et al</i> <sup>[62]</sup>	Yemen	2004	498	Prevalence of T2DM: 7.4%	FPG
Gunaid <i>et al</i> <sup>[63]</sup>	Yemen	2008	250	Prevalence of T2DM: 10.4%	FPG and OGTT
Abdul-Rahim <i>et al</i> <sup>[23]</sup>	Palestine	2001	302	Prevalence of T2DM: 12%	OGTT
Ajlouni <i>et al</i> <sup>[64]</sup>	Jordan	2008	1121	Prevalence of T2DM: 17.4%	FPG
Albache <i>et al</i> <sup>[65]</sup>	Syria	2010	806	Prevalence of T2DM: 15.6%	FPG
Hirbli <i>et al</i> <sup>[66]</sup>	Lebanon	2005	3000	Prevalence of T2DM: 15.6%	FPG
Herman <i>et al</i> <sup>[67]</sup>	Egypt	1998	1451	Prevalence of T2DM: 9.3%	OGTT
Abolfotouh <i>et al</i> <sup>[68]</sup>	Egypt	2008	1800	Prevalence of T2DM: 3.7%	FBG
Elbagir <i>et al</i> <sup>[25]</sup>	Sudan	1996	1284	Prevalence of T2DM: 3.4%	OGTT
Noor <i>et al</i> <sup>[69]</sup>	Sudan	2015	1111	Prevalence of T2DM: 1.3% Prevalence of undiagnosed T2DM: 2.6%	FBG
Bouguerra <i>et al</i> <sup>[26]</sup>	Tunisia	2007	3729	Prevalence of T2DM: 9.9%	FPG
Ben Romdhane <i>et al</i> <sup>[70]</sup>	Tunisia	2014	7700	Prevalence of T2DM: 15.1%	FPG
Kadiki <i>et al</i> <sup>[71]</sup>	Libya	2001	868	Prevalence of T2DM: 14.1%	OGTT
Rguibi <i>et al</i> <sup>[72]</sup>	Morocco	2006	249	Prevalence of undiagnosed T2DM: 6.4%	FPG
Bos <i>et al</i> <sup>[29]</sup>	North Africa	2013	NA	Prevalence of diabetes: Range from 2.6% in rural Sudan to 20.0% in urban Egypt Prevalence of diabetes significantly higher in urban than rural areas Significantly higher prevalence of overweight/obesity in females than males in Algeria, Egypt, Morocco, Tunisia and Sudan	NA
Jaber <i>et al</i> <sup>[73]</sup>	United States (Arab-Americans)	2003	542	Prevalence of T2DM in males: 22.0% Prevalence T2DM in females: 18.0%	OGTT
Rissel <i>et al</i> <sup>[74]</sup>	Australia (Arab immigrants)	1998	528	Prevalence of overweight or obesity in males: 73% Prevalence of overweight or obesity in females: 36%	NA
Thow <i>et al</i> <sup>[75]</sup>	Australia (people born in Middle East and North Africa)	2005	NA	Highest prevalence and incidence of T2DM Second highest ratio of hospitalization and mortality Standard prevalence ratio for diabetes among Arabic-speaking subjects significantly 3.6 times higher than English-only speaking subjects	NA

WHO: World Health Organization; OGTT: Oral glucose tolerance test; FBG: Fasting blood glucose; FPG: Fasting plasma glucose; UAE: United Arab Emirates; NA: Data not available; T2DM: Type-2 diabetes mellitus.

**Table 2** Prevalence of type-2 diabetes mellitus in Arab podiatric and children

Ref.	Country	Year	Sample size	Main findings	Diagnostic technique	Notes
Punnose <i>et al</i> <sup>[76]</sup>	UAE	2005	96	11 children diagnosed with T2DM 9/11 children were Arab origin 8/11 children were overweight or obese 10/11 children were female	FPG	(8-18 years old) Case series
Moussa <i>et al</i> <sup>[6]</sup>	Kuwait	2008	128918	T2DM found in 45 children Prevalence of T2DM in male children: 47.3/10000 Prevalence of T2DM in female children: 26.3/10000 ( $P = 0.05$ )	NA	(6-18 years old) Medical record review
Al-Agha <i>et al</i> <sup>[77]</sup>	Saudi Arabia	2012	387	Prevalence of T2DM: 9.04%	NA	(2-18 years old) Retrospective cross-sectional study
Al-Rubeaan <i>et al</i> <sup>[7]</sup>	Saudi Arabia	2015	23523	Age adjusted Prevalence of T2DM: 1/1000	FPG	$\leq 18$ yr
Ali <i>et al</i> <sup>[78]</sup>	Egypt	2013	210	28 out of 210 children with diabetes diagnosed with T2DM 64.3% of T2DM children were female ( $P = 0.04$ )	Fasting serum C-peptide levels	(1-18 years old)
Osman <i>et al</i> <sup>[79]</sup>	Sudan	2013	958	38/985 children identified with T2DM 32/38 of cases were from tribes of Arab origin	NA	(11-18 years old) Retrospective cross sectional
Ehtisham <i>et al</i> <sup>[80]</sup>	United Kingdom	2000	8	First 8 cases reported with T2DM in United Kingdom All cases were overweight and originated from India, Pakistan and Arab countries	NA	(9-16 years old) Retrospective cross sectional

UAE: United Arab Emirates; FPG: Fasting plasma glucose; NA: Data not available; T2DM: Type-2 diabetes mellitus.

improved medical services, access to education and other “modern” conveniences. There are significant differences in the rate of T2DM between rural and urban communities. For example, an early study in Saudi Arabia found that the prevalence of T2DM in urban communities was 12% and 14% in males and females respectively, which was nearly double the prevalence of T2DM in males and females residing rural areas (7% and 7.7% for males and females respectively)<sup>[28]</sup>. Another study in Oman indicated that urban residence was significantly associated with T2DM (OR = 1.7, 95%CI: 1.4-2.1), with the prevalence of T2DM was 17.7% and 10.5 in urban and rural areas respectively<sup>[21]</sup>. Moreover, a systematic review of the prevalence of T2DM in North Africa found that the prevalence of T2DM ranged from 2.6% in rural Sudan to 20.0% in urban Egypt<sup>[29]</sup>.

### Dietary habits

The Mediterranean diet is considered to be one of the healthiest food options available, as it contains a variety of fruits, vegetables, grains and olive oil. In fact, several studies have shown significant reduction in the rate of T2DM with the Mediterranean diet<sup>[30,31]</sup>. However, one is unlikely to obtain the health benefits of the Mediterranean diet without proper adherence, which may be a common habit in most of the Eastern Mediterranean countries. In a comparative risk assessment analysis, data from the United Food and Agricultural Organization was used to estimate the dietary intake of 20 countries in the Middle East and North Africa<sup>[32]</sup>. These estimates were used to provide a country specific estimates of cardio-metabolic disease mortality secondary to 15 different dietary and metabolic risk factors. This analysis shows that there is suboptimal intake of the “protective” diets (*e.g.*, fruits, vegetables

and sea food), and greater consumption of “harmful” diets (*e.g.*, processed meat and trans fatty acids). These results were reflected in the cardio-metabolic disease mortality rate, where non-optimal BMI was the second leading metabolic risk factor for cardio-metabolic disease mortality, accounting for 21% of all cardio-metabolic mortality risk factors, followed by high fasting blood glucose ( $> 5.3$  mmol/L) which accounted for 17% of all cardio-metabolic disease deaths.

### Sedentary life style

Numerous studies confirm that physical activity reduces the incidence and/or severity of T2DM<sup>[33-35]</sup>. Six years of leading an active life coupled with a healthy diet can reduce the incidence of T2DM by 43% in subjects with impaired glucose tolerance followed for 20 years<sup>[36]</sup>. Another meta-analysis shows that exercise training reduces glycosylated haemoglobin (HbA1c) by 0.66% in type-2 diabetic patients, a percentage that should substantially reduce the complications of T2DM<sup>[37]</sup>. Not surprisingly, a sedentary life style is one of the most important modifiable risk factors in the Arab world, specifically when comparing the prevalence of highly active subjects in the Arab world with the global data. Among 52746 subject from 20 countries included to study the prevalence of physical activity, 8 countries reported that more than 50% of the population are highly active based on the international physical activity questionnaire<sup>[38]</sup>. Saudi Arabia, which was the only country from the Arab world that was included in their analysis, is reported to have 26.2% of their population as being highly active. More recent data came from a study over 10 Eastern Mediterranean countries indicating that the highest level of physically active adolescents were in the Emirates (23.9%), while the lowest was in Egypt

**Table 3** Diabetes complications in the Arab world

Country	Year	Sample size	Prevalence of complication	Ref.
Saudi Arabia	2015	50464	Retinopathy: 19.7%	[81]
	2015	3800	Blindness: 33%	[82]
	2014	54670	Nephropathy: 10.8%	[83]
	2015	62681	Diabetic foot: 3.3%	[84]
			Foot ulcer: 2.05%	
			Gangrene: 0.19%	
			Amputation: 1.06%	
	2014	552	Peripheral neuropathy: 19.9%	[85]
Kuwait	2007	165	Retinopathy: 40%	[86]
Emirates	2007	513	Retinopathy: 19%	[87]
	2007	2455	Retinopathy: 54.2%	[57]
			Nephropathy: 40.8%	
			Neuropathy: 34.7%	
			Peripheral vascular disease: 11.1%	
Bahrain	2009	712	Microalbuminuria: 27.9%	[88]
	2007	1477	Neuropathy: 36.6%	[89]
			Foot ulcer: 5.9%	
			Peripheral vascular disease: 11.8%	
Qatar	2011	540	Retinopathy: 23.5%	[90]
	2014	1633	Retinopathy: 12.5%	[91]
			Nephropathy: 12.4%	
			Neuropathy: 9.5%	
Oman	2003	2249	Retinopathy: 14.9%	[92]
	2009	418	Retinopathy: 7.9%	[93]
	2012	2551	Microalbuminuria: 37%	[94]
			Nephropathy: 5%	
	2012	699	Nephropathy: 42.5%	[95]
Yemen	2011	694	Blindness: 15.7%	[96]
	2009	350	Retinopathy: 55%	[97]
	1997	1095	Peripheral neuropathy: 40.7%	[98]
	2010	311	Peripheral vascular disease: 9.1%	[99]
Jordan	2015	3638	Blindness: 1.3%	[100]
			Severe visual impairment: 1.82%	
			Correctable visual impairment: 9.49%	
	2008	986	Retinopathy: 64.1%	[101]
	2005	986	Blindness: 7.4%	[102]
	2003	1142	Microalbuminuria: 33%	[103]
			Ulceration: 4%	
			Amputation: 5%	
Egypt	2011	1325	Retinopathy: 20.5%	[104]
	2015	2000	Neuropathy: 29.3%	[105]
			Peripheral vascular disease: 11%	
	1998	4600	Retinopathy: 42%	[67]
			Blindness: 5%	
			Nephropathy: 7%	
			Neuropathy: 22%	
			Foot ulcer: 1%	
Tunisia	2014	2320	Retinopathy: 26.3%	[106]
Libya	2012	260	Retinopathy: 16.2%	[107]
			Nephropathy: 1.5%	
			Neuropathy: 11.2%	

(9.2%), giving an overall prevalence of physically active adolescents in the Eastern Mediterranean countries of 19%<sup>[39]</sup>.

### Other factors

Several factors can explain the unrestrained raise in the rate of T2DM in the Arabian area. Some are attributed to the Eastern cultural heritage from a hundred of years ago, such as multiple pregnancies and cultural barriers

for women's physical activity. However, despite no changes in the traditional risk factors for T2DM in Arabian area, there is an alarming increase in the prevalence of diabetes, particularly within the last two decades - suggesting that recent lifestyle changes may have greater effect on this crisis. The global change impacts the Arab nations even more dramatically than elsewhere: Temperatures that are already scorching on a regular basis are now increased (higher temperatures for longer periods), even more increases in polluted and dusty air. These conditions combine to further discourage many people - regardless of age or gender - from any kind of outdoor activity. The situation is made even worse by the political instability in many of these countries, which affects access to healthy food and medical care.

## COMPLICATIONS

According to the World Health Organization, diabetes is the 8<sup>th</sup> leading cause of death in the world<sup>[40]</sup>. Data published specifically from the Arab world shows a similar trend to that available globally. A recent analysis indicates that diabetes represents the 5<sup>th</sup> leading cause of death in the Arab world in 2010, compared to it being the 11<sup>th</sup> leading cause of death in 1990<sup>[41]</sup>. Living with T2DM is troublesome as well, as diabetes is the 5<sup>th</sup> leading cause for the disability-adjusted life years in high income Arabic countries in 2010, compared to it being ranked as the 10<sup>th</sup> reason in twenty years earlier<sup>[41]</sup>. The studies summarized in Table 3 summarize the most recent data on the prevalence of common complications (retinopathy, nephropathy, neuropathy and peripheral vascular diseases) in diabetic patients from the Arab world.

## FUTURE DIRECTIONS

The high prevalence of T2DM in Arab nations offers an opportunity to better understand the disease and its treatment. Unfortunately, current research in the Arab nations does not match the level of this health crisis in the area. Large portions of critical data information are unavailable in many countries from the Arab world. For instance, predicting the prevalence of T2DM statistically could be crucial in formulating a strategic plan for combating the disease, which requires comprehensive knowledge about its current burden, for which data is available only from Tunisia and Saudi Arabia. The Tunisian study revealed that the prevalence of T2DM in Tunisia will reach 26.6% in 2027<sup>[42]</sup>. Moreover, it predicts that a 20% reduction in obesity and smoking will yield in a 3.3% reduction in T2DM by 2027. On the other hand, the Saudi study indicates that the prevalence of T2DM in Saudi Arabia could reach 44.1% in 2022, a figure which differs significantly from IDF estimates<sup>[43]</sup>.

It would be important to apply the recommendations on physical activity discussed at conference on Healthy Lifestyles and Non-Communicable Diseases in the Arab World and the Middle East, also called the "Riyadh

declaration<sup>[44]</sup>. To successfully implement the recommendations of the Riyadh declaration, novel research is needed to determine the social determinants for developing diabetes in the Arab world. For instance, a population based longitudinal cohort of 5124 diabetes-free participants in United States revealed that people residing neighborhoods with fewer opportunities for physical activity have nearly double the risk of developing T2DM<sup>[45]</sup>.

Considering the epidemic nature of obesity and T2DM in the Arab world, studies on the ethnic-specific obesity cut-off points for the risk of diabetes are certainly necessary for peoples of the Arab and North African populations. For example, the risk of diabetes increases with BMI values greater than 25 kg/m<sup>2</sup> for South Asians, at 27 kg/m<sup>2</sup> for African-Caribbeans and at 30 kg/m<sup>2</sup> for Europeans<sup>[46]</sup>.

## CONCLUSION

The data obtained from the Arabic world indicates that there is an uncontrolled rise in the prevalence of T2DM over the last two decades, in particular within the Gulf cooperation countries. For example, the prevalence of T2DM in early 1980's was estimated 2.4%<sup>[8]</sup>, and then increased to 12% in the late 1990's<sup>[28]</sup>, while recent data from Saudi Arabia shows that the prevalence of T2DM reached 25.4% in 2014. Given that obesity is a major risk factor for developing T2DM, obesity is associated with T2DM in Arab countries even where the prevalence of T2DM is relatively low. In addition, other factors such as rapid urbanization, unhealthy dietary habits and the lack of physical activity are key determinants of T2DM in the area. With this uncontrolled rise in the rate of T2DM in the Arab world, T2DM has now become the 5<sup>th</sup> leading cause of death in the Arab world. To better estimate the size of this crisis, studies aimed at predicting the rate of T2DM in the future are urgently needed. However, the vast majority of Arabian countries do not provide this important information. In order to successfully contain the uncontrolled rise of T2DM in the Arabian region, one should take advantage of the research conducted in other communities facing similar patterns in the increasing rates of diabetes. For example, genetic studies, ethnic-specific obesity cut-off points for the risk of diabetes studies and community studies to assess the appropriateness of the neighborhoods for physical activity may bring about increased awareness on the epidemic of diabetes sweeping the region-and so help in creating national/regional strategies to successfully limit the widespread firestorm of T2DM ravaging the Arabic region.

## REFERENCES

- 1 **International Diabetes Federation.** IDF Diabetes Atlas, 6th edition. Brussels, Belgium: International Diabetes Federation, 2013. Available from: URL: <http://www.idf.org/diabetesatlas>
- 2 **American Diabetes Association.** Economic costs of diabetes in the U.S. In 2007. *Diabetes Care* 2008; **31**: 596-615 [PMID: 18308683 DOI: 10.2337/dc08-9017]

- 3 **World Health Organization.** The World Health Statistics. 2008. Available from: URL: <http://www.who.int/whosis/whostat/2008/en/index.ht>
- 4 **Al-Maskari F,** El-Sadig M, Nagelkerke N. Assessment of the direct medical costs of diabetes mellitus and its complications in the United Arab Emirates. *BMC Public Health* 2010; **10**: 679 [PMID: 21059202 DOI: 10.1186/1471-2458-10-679]
- 5 **Badran M,** Laher I. Type II Diabetes Mellitus in Arabic-Speaking Countries. *Int J Endocrinol* 2012; **2012**: 902873 [PMID: 22851968 DOI: 10.1155/2012/902873]
- 6 **Moussa MA,** Alsaied M, Abdella N, Refai TM, Al-Sheikh N, Gomez JE. Prevalence of type 2 diabetes mellitus among Kuwaiti children and adolescents. *Med Princ Pract* 2008; **17**: 270-275 [PMID: 18523392 DOI: 10.1159/000129604]
- 7 **Al-Rubeaan K.** National surveillance for type 1, type 2 diabetes and prediabetes among children and adolescents: a population-based study (SAUDI-DM). *J Epidemiol Community Health* 2015; **69**: 1045-1051 [PMID: 26085648 DOI: 10.1136/jech-2015-205710]
- 8 **Bacchus RA,** Bell JL, Madkour M, Kilshaw B. The prevalence of diabetes mellitus in male Saudi Arabs. *Diabetologia* 1982; **23**: 330-332 [PMID: 7141167 DOI: 10.1007/BF00253739]
- 9 **Fatani HH,** Mira SA, el-Zubier AG. Prevalence of diabetes mellitus in rural Saudi Arabia. *Diabetes Care* 1987; **10**: 180-183 [PMID: 3582078 DOI: 10.2337/diacare.10.2.180]
- 10 **Al-Rubeaan K,** Al-Manaa HA, Khoja TA, Ahmad NA, Al-Sharqawi AH, Siddiqui K, Alnaqeb D, Aburishheh KH, Youssef AM, Al-Batel A, Alotaibi MS, Al-Gamdi AA. Epidemiology of abnormal glucose metabolism in a country facing its epidemic: SAUDI-DM study. *J Diabetes* 2015; **7**: 622-632 [PMID: 25266306 DOI: 10.1111/1753-0407]
- 11 **Dabelea D,** Mayer-Davis EJ, Saydah S, Imperatore G, Linder B, Divers J, Bell R, Badaru A, Talton JW, Crume T, Liese AD, Merchant AT, Lawrence JM, Reynolds K, Dolan L, Liu LL, Hamman RF. Prevalence of type 1 and type 2 diabetes among children and adolescents from 2001 to 2009. *JAMA* 2014; **311**: 1778-1786 [PMID: 24794371 DOI: 10.1001/jama.2014.3201]
- 12 **Zeitler P,** Fu J, Tandon N, Nadeau K, Urakami T, Barrett T, Maahs D. ISPAD Clinical Practice Consensus Guidelines 2014. Type 2 diabetes in the child and adolescent. *Pediatr Diabetes* 2014; **15** Suppl 20: 26-46 [PMID: 25182306 DOI: 10.1111/pedi.12179]
- 13 **Abuyassin B,** Laher I. Obesity-linked diabetes in the Arab world: a review. *East Mediterr Health J* 2015; **21**: 420-439 [PMID: 26370001]
- 14 **Sigal RJ,** Kenny GP, Wasserman DH, Castaneda-Sceppa C. Physical activity/exercise and type 2 diabetes. *Diabetes Care* 2004; **27**: 2518-2539 [PMID: 15451933 DOI: 10.2337/diacare.27.10.2518]
- 15 **Bonora E,** Targher G, Formentini G, Calcaterra F, Lombardi S, Marini F, Zenari L, Saggiani F, Poli M, Perbellini S, Raffaelli A, Gemma L, Santi L, Bonadonna RC, Muggeo M. The Metabolic Syndrome is an independent predictor of cardiovascular disease in Type 2 diabetic subjects. Prospective data from the Verona Diabetes Complications Study. *Diabet Med* 2004; **21**: 52-58 [PMID: 14706054 DOI: 10.1046/j.1464-5491.2003.01068.x]
- 16 **Masuo K,** Rakugi H, Ogiwara T, Esler MD, Lambert GW. Cardiovascular and renal complications of type 2 diabetes in obesity: role of sympathetic nerve activity and insulin resistance. *Curr Diabetes Rev* 2010; **6**: 58-67 [PMID: 20034369 DOI: 10.2174/157339910790909396]
- 17 **Tobias DK,** Pan A, Jackson CL, O'Reilly EJ, Ding EL, Willett WC, Manson JE, Hu FB. Body-mass index and mortality among adults with incident type 2 diabetes. *N Engl J Med* 2014; **370**: 233-244 [PMID: 24428469 DOI: 10.1056/NEJMoa1304501]
- 18 **Logue J,** Walker JJ, Leese G, Lindsay R, McKnight J, Morris A, Philip S, Wild S, Sattar N. Association between BMI measured within a year after diagnosis of type 2 diabetes and mortality. *Diabetes Care* 2013; **36**: 887-893 [PMID: 23139375 DOI: 10.2337/dc12-0944]
- 19 **Al-Shahrani A,** Al-Khaldi Y. Obesity among diabetic and



- hypertensive patients in Aseer region, Saudi Arabia. *Saudi J Obes* 2013; **1**: 14-17 [DOI: 10.4103/2347-2618.119470]
- 20 **Alarouj M**, Bennakhi A, Alnesef Y, Sharifi M, Elcum N. Diabetes and associated cardiovascular risk factors in the State of Kuwait: the first national survey. *Int J Clin Pract* 2013; **67**: 89-96 [PMID: 23241053 DOI: 10.1111/ijcp.12064]
- 21 **Al-Moosa S**, Allin S, Jemai N, Al-Lawati J, Mossialos E. Diabetes and urbanization in the Omani population: an analysis of national survey data. *Popul Health Metr* 2006; **4**: 5 [PMID: 16635266 DOI: 10.1186/1478-7954-4-5]
- 22 **Bener A**, Zirie M, Janahi IM, Al-Hamaq AO, Musallam M, Wareham NJ. Prevalence of diagnosed and undiagnosed diabetes mellitus and its risk factors in a population-based study of Qatar. *Diabetes Res Clin Pract* 2009; **84**: 99-106 [PMID: 19261345 DOI: 10.1016/j.diabres.2009.02.003]
- 23 **Abdul-Rahim HF**, Hussein A, Giacaman R, Jervell J, Bjertness E. Diabetes mellitus in an urban Palestinian population: prevalence and associated factors. *East Mediterr Health J* 2001; **7**: 67-78 [PMID: 12596954]
- 24 **Naja F**, Hwalla N, Itani L, Salem M, Azar ST, Zeidan MN, Nasreddine L. Dietary patterns and odds of Type 2 diabetes in Beirut, Lebanon: a case-control study. *Nutr Metab (Lond)* 2012; **9**: 111 [PMID: 23270372 DOI: 10.1186/1743-7075-9-111]
- 25 **Elbagir MN**, Eltom MA, Elmahadi EM, Kadam IM, Berne C. A population-based study of the prevalence of diabetes and impaired glucose tolerance in adults in northern Sudan. *Diabetes Care* 1996; **19**: 1126-1128 [PMID: 8886561 DOI: 10.2337/diacare.19.10.1126]
- 26 **Bouguerra R**, Alberti H, Salem LB, Rayana CB, Atti JE, Gaigi S, Slama CB, Zouari B, Alberti K. The global diabetes pandemic: the Tunisian experience. *Eur J Clin Nutr* 2007; **61**: 160-165 [PMID: 16900086 DOI: 10.1038/sj.ejcn.1602478]
- 27 **Kahan D**. Prevalence and correlates of adult overweight in the Muslim world: analysis of 46 countries. *Clin Obes* 2015; **5**: 87-98 [PMID: 25755091 DOI: 10.1111/cob.12089]
- 28 **Al-Nuaim AR**. Prevalence of glucose intolerance in urban and rural communities in Saudi Arabia. *Diabet Med* 1997; **14**: 595-602 [PMID: 9223399 DOI: 10.1002/(SICI)1096-9136(199707)14:7<595::AID-DIA377>3.0.CO;2-C]
- 29 **Bos M**, Agyemang C. Prevalence and complications of diabetes mellitus in Northern Africa, a systematic review. *BMC Public Health* 2013; **13**: 387 [PMID: 23617762 DOI: 10.1186/1471-2458-13-387]
- 30 **Martínez-González MA**, de la Fuente-Arrillaga C, Nunez-Cordoba JM, Basterra-Gortari FJ, Beunza JJ, Vazquez Z, Benito S, Tortosa A, Bes-Rastrollo M. Adherence to Mediterranean diet and risk of developing diabetes: prospective cohort study. *BMJ* 2008; **336**: 1348-1351 [PMID: 18511765 DOI: 10.1136/bmj.39561.501007.BE]
- 31 **Salas-Salvadó J**, Bulló M, Babio N, Martínez-González MÁ, Ibarrola-Jurado N, Basora J, Estruch R, Covas MI, Corella D, Arós F, Ruiz-Gutiérrez V, Ros E. Reduction in the incidence of type 2 diabetes with the Mediterranean diet: results of the PREDIMED-Reus nutrition intervention randomized trial. *Diabetes Care* 2011; **34**: 14-19 [PMID: 20929998 DOI: 10.2337/dc10-1288]
- 32 **Afshin A**, Micha R, Khatibzadeh S, Fahimi S, Shi P, Powles J, Singh G, Yakoob MY, Abdollahi M, Al-Hooti S, Farzadfar F, Houshiar-Rad A, Hwalla N, Koksai E, Musaiger A, Pekcan G, Sibai AM, Zaghoul S, Danaei G, Ezzati M, Mozaffarian D. The impact of dietary habits and metabolic risk factors on cardiovascular and diabetes mortality in countries of the Middle East and North Africa in 2010: a comparative risk assessment analysis. *BMJ Open* 2015; **5**: e006385 [PMID: 25995236 DOI: 10.1136/bmjopen-2014-006385]
- 33 **Sigal RJ**, Armstrong MJ, Colby P, Kenny GP, Plotnikoff RC, Reichert SM, Riddell MC. Physical activity and diabetes. *Can J Diabetes* 2013; **37** Suppl 1: S40-S44 [PMID: 24070962 DOI: 10.1016/j.cjcd.2013.01.018]
- 34 **LaMonte MJ**, Blair SN, Church TS. Physical activity and diabetes prevention. *J Appl Physiol* (1985) 2005; **99**: 1205-1213 [PMID: 16103523]
- 35 **Sigal RJ**, Kenny GP, Wasserman DH, Castaneda-Sceppa C, White RD. Physical activity/exercise and type 2 diabetes: a consensus statement from the American Diabetes Association. *Diabetes Care* 2006; **29**: 1433-1438 [PMID: 16732040 DOI: 10.2337/dc06-9910]
- 36 **Li G**, Zhang P, Wang J, Gregg EW, Yang W, Gong Q, Li H, Li H, Jiang Y, An Y, Shuai Y, Zhang B, Zhang J, Thompson TJ, Gerzoff RB, Roglic G, Hu Y, Bennett PH. The long-term effect of lifestyle interventions to prevent diabetes in the China Da Qing Diabetes Prevention Study: a 20-year follow-up study. *Lancet* 2008; **371**: 1783-1789 [PMID: 18502303 DOI: 10.1016/S0140-6736(08)6076-6-7]
- 37 **Boulé NG**, Haddad E, Kenny GP, Wells GA, Sigal RJ. Effects of exercise on glycemic control and body mass in type 2 diabetes mellitus: a meta-analysis of controlled clinical trials. *JAMA* 2001; **286**: 1218-1227 [PMID: 11559268]
- 38 **Bauman A**, Bull F, Chey T, Craig CL, Ainsworth BE, Sallis JF, Bowles HR, Hagstromer M, Sjostrom M, Pratt M. The International Prevalence Study on Physical Activity: results from 20 countries. *Int J Behav Nutr Phys Act* 2009; **6**: 21 [PMID: 19335883 DOI: 10.1186/1479-5868-6-21]
- 39 **Al Subhi LK**, Bose S, Al Ani MF. Prevalence of physically active and sedentary adolescents in 10 Eastern Mediterranean countries and its relation with age, sex, and body mass index. *J Phys Act Health* 2015; **12**: 257-265 [PMID: 24763105 DOI: 10.1123/jpah.2013-0134]
- 40 **World Health Organization**. Fact sheet No. 310. 2014. Available from: URL: <http://www.who.int/mediacentre/factsheets/fs310/en>
- 41 **Mokdad AH**, Jaber S, Aziz MI, AlBuhairan F, AlGhaithi A, AlHamad NM, Al-Hooti SN, Al-Jasari A, AlMazroa MA, AlQasbi AM, Alswaidi S, Asad M, Atkinson C, Badawi A, Bakfalouni T, Barkia A, Biryukov S, El Bcheraoui C, Daoud F, Forouzanfar MH, Gonzalez-Medina D, Hamadeh RH, Hsaio R, Hussein SS, Karam N, Khalifa SE, Khoja TA, Lami F, Leach-Kemon K, Memish ZA, Mokdad AA, Naghavi M, Nasher J, Qasem MB, Shuaib M, Al Thani AA, Al Thani MH, Zamakhshary M, Lopez AD, Murray CJ. The state of health in the Arab world, 1990-2010: an analysis of the burden of diseases, injuries, and risk factors. *Lancet* 2014; **383**: 309-320 [PMID: 24452042 DOI: 10.1016/S0140-6736(13)62189-3]
- 42 **Saidi O**, O'Flaherty M, Mansour NB, Aissi W, Lassoued O, Capewell S, Critchley JA, Malouche D, Romdhane HB. Forecasting Tunisian type 2 diabetes prevalence to 2027: validation of a simple model. *BMC Public Health* 2015; **15**: 104 [PMID: 25885910 DOI: 10.1186/s12889-015-1416-z]
- 43 **Al-Quwaidhi AJ**, Pearce MS, Sobngwi E, Critchley JA, O'Flaherty M. Comparison of type 2 diabetes prevalence estimates in Saudi Arabia from a validated Markov model against the International Diabetes Federation and other modelling studies. *Diabetes Res Clin Pract* 2014; **103**: 496-503 [PMID: 24447810 DOI: 10.1016/j.diabres.2013.12.036]
- 44 **Bull F**, Dvorak J. Tackling chronic disease through increased physical activity in the Arab World and the Middle East: challenge and opportunity. *Br J Sports Med* 2013; **47**: 600-602 [PMID: 23624465 DOI: 10.1136/bjsports-2012-092109]
- 45 **Christine PJ**, Auchincloss AH, Bertoni AG, Carnethon MR, Sánchez BN, Moore K, Adar SD, Horwich TB, Watson KE, Diez Roux AV. Longitudinal Associations Between Neighborhood Physical and Social Environments and Incident Type 2 Diabetes Mellitus: The Multi-Ethnic Study of Atherosclerosis (MESA). *JAMA Intern Med* 2015; **175**: 1311-1320 [PMID: 26121402 DOI: 10.1001/jamainternmed.2015.2691]
- 46 **Tillin T**, Sattar N, Godsland IF, Hughes AD, Chaturvedi N, Forouhi NG. Ethnicity-specific obesity cut-points in the development of Type 2 diabetes - a prospective study including three ethnic groups in the United Kingdom. *Diabet Med* 2015; **32**: 226-234 [PMID: 25186015 DOI: 10.1111/dme.12576]
- 47 **Rahman Al-Nuaim A**. Effect of overweight and obesity on glucose intolerance and dyslipidemia in Saudi Arabia, epidemiological study. *Diabetes Res Clin Pract* 1997; **36**: 181-191 [PMID: 9237785 DOI: 10.1016/S0168-8227(97)00041-7]
- 48 **el-Hazmi MA**, Wasy AS. Prevalence of overweight and obesity in diabetic and non-diabetic Saudis. *East Mediterr Health J* 2000; **6**:

- 276-282 [PMID: 11556013]
- 49 **Al-Nozha MM**, Al-Maatouq MA, Al-Mazrou YY, Al-Harthi SS, Arafah MR, Khalil MZ, Khan NB, Al-Khadra A, Al-Marzouki K, Nouh MS, Abdullah M, Attas O, Al-Shahid MS, Al-Mobeireek A. Diabetes mellitus in Saudi Arabia. *Saudi Med J* 2004; **25**: 1603-1610 [PMID: 15573186]
- 50 **Almajwal AM**, Al-Baghli NA, Batterham MJ, Williams PG, Al-Turki KA, Al-Ghamdi AJ. Performance of body mass index in predicting diabetes and hypertension in the Eastern Province of Saudi Arabia. *Ann Saudi Med* 2009; **29**: 437-445 [PMID: 19847080 DOI: 10.4103/0256-4947.57165]
- 51 **Al-Rubeaan K**, Al-Manaa H, Khoja T, Ahmad N, Al-Sharqawi A, Siddiqui K, AlNaqeb D, Aburishheh K, Youssef A, Al-Batil A, Al-Otaibi M, Ghamdi AA. The Saudi Abnormal Glucose Metabolism and Diabetes Impact Study (SAUDI-DM). *Ann Saudi Med* 2014; **34**: 465-475 [PMID: 25971818 DOI: 10.5144/0256-4947.2014.465]
- 52 **El Bcheraoui C**, Basulaiman M, Tuffaha M, Daoud F, Robinson M, Jaber S, Mikhitarian S, Memish ZA, Al Saeedi M, AlMazroa MA, Mokdad AH. Status of the diabetes epidemic in the Kingdom of Saudi Arabia, 2013. *Int J Public Health* 2014; **59**: 1011-1021 [PMID: 25292457 DOI: 10.1007/s00038-014-0612-4]
- 53 **Abdella N**, Khogali M, al-Ali S, Gumaa K, Bajaj J. Known type 2 diabetes mellitus among the Kuwaiti population. A prevalence study. *Acta Diabetol* 1996; **33**: 145-149 [PMID: 8870817 DOI: 10.1007/BF00569425]
- 54 **Al Khalaf MM**, Eid MM, Najjar HA, Alhajry KM, Doi SA, Thalib L. Screening for diabetes in Kuwait and evaluation of risk scores. *East Mediterr Health J* 2010; **16**: 725-731 [PMID: 20799528]
- 55 **Al Zurba FI**, Al Garf A. Prevalence of diabetes mellitus among Bahrainis attending primary health care centres. *East Mediterr Health J* 1996; **2**: 274-282
- 56 **Malik M**, Bakir A, Saab BA, King H. Glucose intolerance and associated factors in the multi-ethnic population of the United Arab Emirates: results of a national survey. *Diabetes Res Clin Pract* 2005; **69**: 188-195 [PMID: 16005369 DOI: 10.1016/j.diabres.2004.12.005]
- 57 **Saadi H**, Carruthers SG, Nagelkerke N, Al-Maskari F, Afandi B, Reed R, Lukic M, Nicholls MG, Kazam E, Algawi K, Al-Kaabi J, Leduc C, Sabri S, El-Sadig M, Elkhumaidi S, Agarwal M, Benedict S. Prevalence of diabetes mellitus and its complications in a population-based sample in Al Ain, United Arab Emirates. *Diabetes Res Clin Pract* 2007; **78**: 369-377 [PMID: 17532085 DOI: 10.1016/j.diabres.2007.04.008]
- 58 **Mansour AA**, Al-Jazairi MI. Predictors of incident diabetes mellitus in Basrah, Iraq. *Ann Nutr Metab* 2007; **51**: 277-280 [PMID: 17622787 DOI: 10.1159/000105449]
- 59 **Mansour AA**, Al-Maliky AA, Kasem B, Jabar A, Mosbeh KA. Prevalence of diagnosed and undiagnosed diabetes mellitus in adults aged 19 years and older in Basrah, Iraq. *Diabetes Metab Syndr Obes* 2014; **7**: 139-144 [PMID: 24833912 DOI: 10.2147/DMSO.S59652]
- 60 **Al-Lawati JA**, Panduranga P, Al-Shaikh HA, Morsi M, Mohsin N, Khandekar RB, Al-Lawati HJ, Bayoumi RA. Epidemiology of Diabetes Mellitus in Oman: Results from two decades of research. *Sultan Qaboos Univ Med J* 2015; **15**: e226-e233 [PMID: 26052456]
- 61 **Musaiger A**, Shahbeek N. The relationship between obesity and prevalence of chronic diseases in the Arab women. *J Hum Ecol Special Issue* 2005; **13**: 97-100
- 62 **Al-Habori M**, Al-Mamari M, Al-Meer A. Type II Diabetes Mellitus and impaired glucose tolerance in Yemen: prevalence, associated metabolic changes and risk factors. *Diabetes Res Clin Pract* 2004; **65**: 275-281 [PMID: 15331208 DOI: 10.1016/j.diabres.2004.02.001]
- 63 **Gunaid AA**, Assabri AM. Prevalence of type 2 diabetes and other cardiovascular risk factors in a semirural area in Yemen. *East Mediterr Health J* 2008; **14**: 42-56 [PMID: 18557451]
- 64 **Ajlouni K**, Khader YS, Batieha A, Ajlouni H, El-Khateeb M. An increase in prevalence of diabetes mellitus in Jordan over 10 years. *J Diabetes Complications* 2008; **22**: 317-324 [PMID: 18413210 DOI: 10.1016/j.jdiacomp.2007.01.004]
- 65 **Albache N**, Al Ali R, Rastam S, Fouad FM, Mzayek F, Maziak W. Epidemiology of Type 2 diabetes mellitus in Aleppo, Syria. *J Diabetes* 2010; **2**: 85-91 [PMID: 20923489 DOI: 10.1111/j.1753-0407.2009.00063]
- 66 **Hirbli KI**, Jambeine MA, Slim HB, Barakat WM, Habis RJ, Francis ZM. Prevalence of diabetes in greater Beirut. *Diabetes Care* 2005; **28**: 1262 [PMID: 15855610 DOI: 10.2337/diacare.28.5.1262]
- 67 **Herman WH**, Aubert RE, Engelgau MM, Thompson TJ, Ali MA, Sous ES, Hegazy M, Badran A, Kenny SJ, Gunter EW, Malarcher AM, Brechner RJ, Wetterhall SF, DeStefano F, Smith PJ, Habib M, abd el Shakour S, Ibrahim AS, el Behairy EM. Diabetes mellitus in Egypt: glycaemic control and microvascular and neuropathic complications. *Diabet Med* 1998; **15**: 1045-1051 [PMID: 9868980 DOI: 10.1002/(SICI)1096-9136(1998120)15:12<1045::AID-DIA696>3.0.CO;2-L]
- 68 **Abolfotouh MA**, Soliman LA, Mansour E, Farghaly M, El-Dawaiaty AA. Central obesity among adults in Egypt: prevalence and associated morbidity. *East Mediterr Health J* 2008; **14**: 57-68 [PMID: 18557452]
- 69 **Noor SK**, Bushara SO, Sulaiman AA, Elmadhoun WM, Ahmed MH. Undiagnosed diabetes mellitus in rural communities in Sudan: prevalence and risk factors. *East Mediterr Health J* 2015; **21**: 164-170 [PMID: 26074216]
- 70 **Ben Romdhane H**, Ben Ali S, Aissi W, Traissac P, Aounallah-Skhiri H, Bougateg S, Maire B, Delpuech F, Achour N. Prevalence of diabetes in Northern African countries: the case of Tunisia. *BMC Public Health* 2014; **14**: 86 [PMID: 24472619 DOI: 10.1186/1471-2458-14-86]
- 71 **Kadiki OA**, Roaeid RB. Prevalence of diabetes mellitus and impaired glucose tolerance in Benghazi Libya. *Diabetes Metab* 2001; **27**: 647-654 [PMID: 11852372]
- 72 **Rguibi M**, Belahsen R. Prevalence and associated risk factors of undiagnosed diabetes among adult Moroccan Sahraoui women. *Public Health Nutr* 2006; **9**: 722-727 [PMID: 16925877 DOI: 10.1079/PHN2005866]
- 73 **Jaber LA**, Brown MB, Hammad A, Nowak SN, Zhu Q, Ghafoor A, Herman WH. Epidemiology of diabetes among Arab Americans. *Diabetes Care* 2003; **26**: 308-313 [PMID: 12547854]
- 74 **Rissel C**, Lesjak M, Ward J. Cardiovascular risk factors among Arabic-speaking patients attending Arabic-speaking general practitioners in Sydney, Australia: opportunities for intervention. *Ethn Health* 1998; **3**: 213-222 [PMID: 9798119 DOI: 10.1080/1357858.1998.9961863]
- 75 **Thow AM**, Waters AM. AIHW 2005. Diabetes in culturally and linguistically diverse Australians. Cat. No. CVD 30. Canberra: Australian Institute of Health and Welfare, 2005
- 76 **Punnose J**, Agarwal MM, Bin-Uthman S. Type 2 diabetes mellitus among children and adolescents in Al-Ain: a case series. *East Mediterr Health J* 2005; **11**: 788-797 [PMID: 16700395]
- 77 **Al-Agha A**, Ocheltree A, Shata N. Prevalence of hyperinsulinism, type 2 diabetes mellitus and metabolic syndrome among Saudi overweight and obese pediatric patients. *Minerva Pediatr* 2012; **64**: 623-631 [PMID: 23108324]
- 78 **Ali BA**, Abdallah ST, Abdallah AM, Hussein MM. The Frequency of Type 2 Diabetes Mellitus among Diabetic Children in El Minia Governorate, Egypt. *Sultan Qaboos Univ Med J* 2013; **13**: 399-403 [PMID: 23984025 DOI: 10.12816/0003262]
- 79 **Osman H**, Elsadek N, Abdullah M. Type 2 diabetes in Sudanese children and adolescents. *Sudan J Paediatr* 2013; **13**: 17-23
- 80 **Ehtisham S**, Barrett TG, Shaw NJ. Type 2 diabetes mellitus in UK children--an emerging problem. *Diabet Med* 2000; **17**: 867-871 [PMID: 11168330 DOI: 10.1046/j.1464-5491.2000.00409.x]
- 81 **Al-Rubeaan K**, Abu El-Asrar AM, Youssef AM, Subhani SN, Ahmad NA, Al-Sharqawi AH, Alguwaihes A, Alotaibi MS, Al-Ghamdi A, Ibrahim HM. Diabetic retinopathy and its risk factors in a society with a type 2 diabetes epidemic: a Saudi National Diabetes Registry-based study. *Acta Ophthalmol* 2015; **93**: e140-e147 [PMID: 25270515 DOI: 10.1111/aos.12532]
- 82 **Hajar S**, Al Hazmi A, Wasli M, Mousa A, Rabiou M. Prevalence

- and causes of blindness and diabetic retinopathy in Southern Saudi Arabia. *Saudi Med J* 2015; **36**: 449-455 [PMID: 25828282 DOI: 10.15537/smj.2015.4.10371]
- 83 **Al-Rubeaan K**, Youssef AM, Subhani SN, Ahmad NA, Al-Sharqawi AH, Al-Mutlaq HM, David SK, AlNaqeb D. Diabetic nephropathy and its risk factors in a society with a type 2 diabetes epidemic: a Saudi National Diabetes Registry-based study. *PLoS One* 2014; **9**: e88956 [PMID: 24586457 DOI: 10.1371/journal.pone.0088956]
  - 84 **Al-Rubeaan K**, Al Derwish M, Ouizi S, Youssef AM, Subhani SN, Ibrahim HM, Alamri BN. Diabetic foot complications and their risk factors from a large retrospective cohort study. *PLoS One* 2015; **10**: e0124446 [PMID: 25946144 DOI: 10.1371/journal.pone.0124446]
  - 85 **Wang DD**, Bakhotmah BA, Hu FB, Alzahrani HA. Prevalence and correlates of diabetic peripheral neuropathy in a Saudi Arabic population: a cross-sectional study. *PLoS One* 2014; **9**: e106935 [PMID: 25184511 DOI: 10.1371/journal.pone.0106935]
  - 86 **Al-Adsani AM**. Risk factors for diabetic retinopathy in Kuwaiti type 2 diabetic patients. *Saudi Med J* 2007; **28**: 579-583 [PMID: 17457481]
  - 87 **Al-Maskari F**, El-Sadig M. Prevalence of diabetic retinopathy in the United Arab Emirates: a cross-sectional survey. *BMC Ophthalmol* 2007; **7**: 11 [PMID: 17572909 DOI: 10.1186/1471-2415-7-11]
  - 88 **Al-Salman RA**, Al-Basri HA, Al-Sayyad AS, Hearnshaw HM. Prevalence and risk factors of albuminuria in Type 2 diabetes in Bahrain. *J Endocrinol Invest* 2009; **32**: 746-751 [PMID: 20009501 DOI: 10.1007/BF03346530]
  - 89 **Al-Mahroos F**, Al-Roomi K. Diabetic neuropathy, foot ulceration, peripheral vascular disease and potential risk factors among patients with diabetes in Bahrain: a nationwide primary care diabetes clinic-based study. *Ann Saudi Med* 2007; **27**: 25-31 [PMID: 17277500 DOI: 10.4103/0256-4947.51536]
  - 90 **Bener A**, Al-Laftah F, Al-Hamaq AO, Daghash M, Abdullatef WK. A study of diabetes complications in an endogamous population: an emerging public health burden. *Diabetes Metab Syndr* 2014; **8**: 108-114 [PMID: 24907176 DOI: 10.1016/j.dsx.2014.04.005]
  - 91 **Elshafei M**, Gamra H, Khandekar R, Al Hashimi M, Pai A, Ahmed MF. Prevalence and determinants of diabetic retinopathy among persons  $\geq 40$  years of age with diabetes in Qatar: a community-based survey. *Eur J Ophthalmol* 2011; **21**: 39-47 [PMID: 20602322 DOI: 10.5301/EJO.2010.2699]
  - 92 **Khandekar R**, Al Lawatii J, Mohammed AJ, Al Raisi A. Diabetic retinopathy in Oman: a hospital based study. *Br J Ophthalmol* 2003; **87**: 1061-1064 [PMID: 12928265]
  - 93 **Khandekar RB**, Tirumurthy S, Al-Harby S, Moorthy NS, Amir I. Diabetic retinopathy and ocular co-morbidities among persons with diabetes at Sumail Hospital of Oman. *Diabetes Technol Ther* 2009; **11**: 675-679 [PMID: 19821761 DOI: 10.1089/dia.2009.0032]
  - 94 **Al-Lawati JA**, N Barakat M, Al-Zakwani I, Elsayed MK, Al-Maskari M, M Al-Lawati N, Mohammed AJ. Control of risk factors for cardiovascular disease among adults with previously diagnosed type 2 diabetes mellitus: a descriptive study from a middle eastern arab population. *Open Cardiovasc Med J* 2012; **6**: 133-140 [PMID: 23166566 DOI: 10.2174/1874192401206010133]
  - 95 **Alrawahi AH**, Rizvi SG, Al-Riyami D, Al-Anqoodi Z. Prevalence and risk factors of diabetic nephropathy in omani type 2 diabetics in Al-dakhiliyah region. *Oman Med J* 2012; **27**: 212-216 [PMID: 22811770 DOI: 10.5001/omj.2012.48]
  - 96 **Al-Akily SA**, Bamashmus MA, Gunaid AA. Causes of visual impairment and blindness among Yemenis with diabetes: a hospital-based study. *East Mediterr Health J* 2011; **17**: 831-837 [PMID: 22276490]
  - 97 **Bamashmus MA**, Gunaid AA, Khandekar RB. Diabetic retinopathy, visual impairment and ocular status among patients with diabetes mellitus in Yemen: a hospital-based study. *Indian J Ophthalmol* 2009; **57**: 293-298 [PMID: 19574698 DOI: 10.4103/0301-4738.53055]
  - 98 **Gunaid AA**, El Khally FM, Hassan NA, Mukhtar el D. Demographic and clinical features of diabetes mellitus in 1095 Yemeni patients. *Ann Saudi Med* 1997; **17**: 402-409 [PMID: 17353590]
  - 99 **Al-Khawlani A**, Atef ZA, Al-Ansi A. Macrovascular complications and their associated risk factors in type 2 diabetic patients in Sana' a city, Yemen. *East Mediterr Health J* 2010; **16**: 851-858 [PMID: 21469567]
  - 100 **Rabiu MM**, Al Bdour MD, Abu Ameerh MA, Jadoon MZ. Prevalence of blindness and diabetic retinopathy in northern Jordan. *Eur J Ophthalmol* 2015; **25**: 320-327 [PMID: 25684158 DOI: 10.5301/ejo.5000557]
  - 101 **Al-Bdour MD**, Al-Till MI, Abu Samra KM. Risk Factors for Diabetic Retinopathy among Jordanian Diabetics. *Middle East Afr J Ophthalmol* 2008; **15**: 77-80 [PMID: 21346842 DOI: 10.4103/0974-9233.51997]
  - 102 **Al-Till MI**, Al-Bdour MD, Ajlouni KM. Prevalence of blindness and visual impairment among Jordanian diabetics. *Eur J Ophthalmol* 2005; **15**: 62-68 [PMID: 15751241]
  - 103 **Jbour AS**, Jarrah NS, Radaideh AM, Shegem NS, Bader IM, Batieha AM, Ajlouni KM. Prevalence and predictors of diabetic foot syndrome in type 2 diabetes mellitus in Jordan. *Saudi Med J* 2003; **24**: 761-764 [PMID: 12883610]
  - 104 **Mackey TA**, Khater N, Al-Zamil MA, El Fishawy H, Soliman MM. Epidemiology of diabetic retinopathy in Egypt: a hospital-based study. *Ophthalmic Res* 2011; **45**: 73-78 [PMID: 20714195 DOI: 10.1159/000314876]
  - 105 **Assaad-Khalil SH**, Zaki A, Abdel Rehman A, Megallaa MH, Gaber N, Gamal H, Rohoma KH. Prevalence of diabetic foot disorders and related risk factors among Egyptian subjects with diabetes. *Prim Care Diabetes* 2015; **9**: 297-303 [PMID: 25543864 DOI: 10.1016/j.pcd.2014.10.010]
  - 106 **Kahloun R**, Jelliti B, Zaouali S, Attia S, Ben Yahia S, Resnikoff S, Khairallah M. Prevalence and causes of visual impairment in diabetic patients in Tunisia, North Africa. *Eye (Lond)* 2014; **28**: 986-991 [PMID: 24924439 DOI: 10.1038/eye.2014.131]
  - 107 **Elhwuegi AS**, Darez AA, Langa AM, Bashaga NA. Cross-sectional pilot study about the health status of diabetic patients in city of Misurata, Libya. *Afr Health Sci* 2012; **12**: 81-86 [PMID: 23066426]

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## Comment on: Statin use and risk of diabetes mellitus

Mehmet Ali Eren, Tefik Sabuncu, Hüseyin Karaaslan

Mehmet Ali Eren, Tefik Sabuncu, Hüseyin Karaaslan,  
 Department of Endocrinology, Harran University, School of  
 Medicine, 63100 Sanliurfa, Turkey

Author contributions: Eren MA and Karaaslan H wrote this  
 letter; Sabuncu T edited and revised this letter.

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Correspondence to: Mehmet Ali Eren, MD, Department  
 of Endocrinology, Harran University, School of Medicine,  
 Valikonagi Street, Yenisehir Campus, 63100 Sanliurfa,  
 Turkey. [drmalieren@hotmail.com](mailto:drmalieren@hotmail.com)  
 Telephone: +90-414-3128456

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### Abstract

In manuscript named "Statin use and risk of diabetes  
 mellitus" by Chogtu *et al*, authors defined that pravastatin  
 40 mg/dL reduced the risk of diabetes by 30% in  
 West of Scotland Coronary Prevention study. In fact,  
 pravastatin 40 mg/dL reduced coronary heart disease  
 risk approximately 30% in mentioned study.

**Key words:** Pravastatin; Statins; Diabetes mellitus;

Coronary heart disease; Myocardial Infarction

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**Core tip:** We want to eliminate an important error that  
 lead to confusion about the risk of diabetes due to statins  
 in the well-written manuscript by Chogtu *et al*.

Eren MA, Sabuncu T, Karaaslan H. Comment on: Statin use and  
 risk of diabetes mellitus. *World J Diabetes* 2016; 7(8): 175-176  
 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v7/i8/175.htm> DOI: <http://dx.doi.org/10.4239/wjd.v7.i8.175>

### TO THE EDITOR

We read with great interest the recent review by  
 Chogtu *et al*<sup>[1]</sup> dealing with the risk of diabetes mellitus  
 development induced by the use of statins. The authors  
 clearly explained the benefit of statin on cardiovascular  
 prevention as well as the possible mechanism of  
 impaired glucose metabolism related with statin. In our  
 opinion, there was a critical confusing error in "statins in  
 diabetes" section.

In the last sentence of mentioned section, authors  
 defined that pravastatin 40 mg/dL reduced the risk  
 of diabetes by 30% in West of Scotland Coronary  
 Prevention study with reference to the Kotseva *et al*<sup>[2]</sup>.  
 However, pravastatin 40 mg/dL reduced nonfatal  
 myocardial infarctions risk by 31%, death from coronary  
 heart disease by 28%, death from all cardiovascular  
 causes by 32% but there was no information about  
 diabetes risk in the original study of West of Scotland  
 Coronary Prevention Study Group<sup>[3]</sup>.

We hope that the correction of above-mentioned  
 item would eliminate the confusion and provide better  
 understanding of the well-written manuscript by Chogtu  
*et al*<sup>[1]</sup>.



## REFERENCES

- 1 **Chogtu B**, Magazine R, Bairy KL. Statin use and risk of diabetes mellitus. *World J Diabetes* 2015; **6**: 352-357 [PMID: 25789118 DOI: 10.4239/wjd.v6.i2.352]
- 2 **Kotseva K**, Wood D, De Backer G, De Bacquer D, Pyörälä K, Keil U. Cardiovascular prevention guidelines in daily practice: a comparison of EUROASPIRE I, II, and III surveys in eight European countries. *Lancet* 2009; **373**: 929-940 [PMID: 19286092 DOI: 10.1016/S0140-6736(09)60330-5]
- 3 **Shepherd J**, Cobbe SM, Ford I, Isles CG, Lorimer AR, MacFarlane PW, McKillop JH, Packard CJ. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. West of Scotland Coronary Prevention Study Group. *N Engl J Med* 1995; **333**: 1301-1307 [PMID: 7566020 DOI: 10.1056/NEJM199511163332001]

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Fax: +86-10-85381893

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## Positive evidence for vitamin A role in prevention of type 1 diabetes

Somaye Yosae, Maryam Akbari Fakhrabadi, Farzad Shidfar

Somaye Yosae, Maryam Akbari Fakhrabadi, Farzad Shidfar, Department of Nutrition, School of Public Health, Iran University of Medical Sciences, Tehran 14665-1579, Iran

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**Correspondence to:** Farzad Shidfar, PhD, Department of Nutrition, School of Public Health, Iran University of Medical Sciences, Shahid Hemmat Expressway, P.O. Box, Tehran 14665-1579, Iran. [shidfar.f@iums.ac.ir](mailto:shidfar.f@iums.ac.ir)  
 Telephone: +98-21-88622755  
 Fax: +98-21-88622533

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### Abstract

Type 1 diabetes mellitus (T1DM) as one of the most well-known autoimmune disease, results from the destruction of  $\beta$ -cells in pancreas by autoimmune process. T1DM is fatal without insulin treatment. The

expansion of alternative treatment to insulin is a dream to be fulfilled. Currently autoimmunity is considered as main factor in development of T1DM. So manipulation of the immune system can be considered as alternative treatment to insulin. For the past decades, vitamin A has been implicated as an essential dietary micronutrient in regulator of immune function. Despite major advantage in the knowledge of vitamin A biology, patients who present T1DM are at risk for deficiency in vitamin A and carotenoids. Applying such evidences, vitamin A treatment may be the key approach in preventing T1DM.

**Key words:** Diabetes; Autoimmune; Pancreas; Insulin; Vitamin A

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**Core tip:** Diet modification and vitamin supplementation is a practical treatment approach for autoimmune diseases. However few broadly studies have been conducted on the use of vitamin A in the treatment of type 1 diabetes. Our objective is to consolidate the current literature to better delineate the vitamin A on immune pathway involved in formation of type 1 diabetes.

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### INTRODUCTION

Type 1 diabetes mellitus (T1DM) is a well-known autoimmune disease that is characterized by a state of T cell-mediated selective deficiency of absolute or relative insulin-producing  $\beta$ -cells<sup>[1-4]</sup>. Despite modern medical management, T1DM is still one of the most



common chronic childhood diseases<sup>[5]</sup>. T1DM eventually ends up in several disorders including renal failure, ketoacidosis, heart disease, stroke and visionless<sup>[1]</sup>. It is estimated that T1DM affects 497100 children under 15 years globally<sup>[5]</sup>. Currently administration of exogenous insulin has been the core strategy of treatment for patient with T1DM<sup>[4,6]</sup>. However it is not without complications. So, alternative preventive and treatment approaches to insulin are required. It has been shown that manipulation of the immune system with altering the course of the disease can be considered as alternative preventive and treatments approach to insulin<sup>[7]</sup>. The effects of vitamin A on immune system have been studied more than any other nutrients<sup>[8]</sup>. The concept of modulation of immune system by vitamin A dates back to the early twenties and the work of Green *et al*<sup>[9,10]</sup> who reported that vitamin A and  $\beta$ -carotene have "anti-infective" properties. Today, dietary vitamin A and its derivatives are recognized as crucial agents for normal immune system function and regulation<sup>[11]</sup>.

Interestingly, recent studies have demonstrated that vitamin A deficiency leads to defects in glucose-stimulated insulin secretion<sup>[12]</sup>. In addition presence of relatively high levels of cellular retinol binding protein (RBP), cellular retinoic acid (RA) binding proteins, transthyretin (TTR) and RBP in pancreatic rat islets have been documented<sup>[13-15]</sup>. Despite major advantage in the knowledge of vitamin A biology, patients who present T1DM are at risk for deficiency in vitamin A and carotenoids<sup>[16,17]</sup>. It has been shown that bioavailability and plasma concentrations of vitamin A, TTR, retinol, and RBP fall in children and adults with T1DM<sup>[18-22]</sup>.

The action of vitamin A to control immune response has led to a growing hypothesis of potential role of vitamin A in T1DM as an autoimmune disease. This review highlights new information regarding to vitamin A and RA in regulation of immune responses in patient with T1DM. So, the purpose of the current study was to thoroughly review the function of vitamin A in the immunology of T1DM.

## RESEARCH

We performed a comprehensive literature search of the subject using MEDLINE and PubMed, for "vitamin A", OR "retinoic acid" OR "retinol" AND "type 1 diabetes mellitus" OR "T1DM". All papers fulfilling the above criteria were considered. All papers obtained in the search were fully discussed by the authors. References lists of all original published articles were scanned to find additional eligible studies.

## VITAMIN A/RA METABOLISM AND SIGNALING

Vitamin A is an essential nutrient that can be acquired from the diet either as preformed vitamin A [primarily as retinyl ester (RE), retinol, and in much smaller amount

as RA] or provitamin A carotenoids<sup>[23]</sup>. This vitamin has been well known for its critical function in embryonic development, vision and the nervous system, as well as in regulation and development of the immune system<sup>[24]</sup>. Both dietary vitamin A as vegetable and fruit-derived carotenoids and REs from animal sources are converted to retinol within the lumen of the small intestine or the intestinal mucosa and then enzymatically re-esterified with long-chain fatty acids within the enterocyte to form RE<sup>[23,25-27]</sup>. REs are packaged into chylomicrons together with other dietary lipids and secreted into the lymphatic system<sup>[27]</sup>. The liver is the primary organ for storage of vitamin A, where the retinol form of vitamin is esterified by lecithin: Retinol acyl transferase and stored as a RE<sup>[28,29]</sup>. To meet the tissue vitamin A needs, retinol released into the circulation from liver and bound to its specific transport protein, retinol-binding protein (RBP or RBP4)<sup>[30,31]</sup>.

In the liver and the peripheral stream, vitamin A is mainly in the form of retinol and REs. Although the function of vitamin A is applied in its metabolite form RA<sup>[28]</sup>. So, its precursors must be converted to RA by a two-step process<sup>[32]</sup>. First, retinol is hydrolyzed into retinal by ubiquitous alcohol dehydrogenase, and then irreversible hydrolysis reaction allows the formation of RA<sup>[33]</sup>. Regulation of gene expression by RA, and the discovery of RA receptor and retinoic acid X receptor which are specific receptors for the active metabolites of vitamin A such as all trans and 9-cis-retinoic acids, provided fundamental documents for the understanding retinoids effects on immune function<sup>[34-36]</sup>. RA is inactivated by CYP26A1, CYP26B1 and CYP26C1<sup>[28,29]</sup>.

## PREDIABETES STAGE

Type 1 diabetes is an autoimmune disease, which result from development of islet autoantibodies against proteins in insulin producing beta cells and immune-mediated destruction of insulin producing beta cells in the pancreas<sup>[37]</sup>. These individuals with antibody positive within many years are at risk of developing T1DM<sup>[38-40]</sup>. Progressive autoimmune  $\beta$ -cell damage usually precedes the clinical onset of diabetes, and occurs years before any clinical symptom of T1DM<sup>[41,42]</sup>. This long pre-diabetes phase making T1DM as a predictable disease, and provides an opportunity to prevent individuals with active insulinitis from developing clinical disease<sup>[37]</sup>.

So T1DM will be a preventable disease by the intervention targeting the manipulating of immune system. In this context one approach is the trimolecular complex, including a self-reactive CD4 T cell, insulin, and HLA molecule<sup>[43]</sup>.

## TYPE 1 DIABETES AND INNATE IMMUNE RESPONSES

The body's first defense system against microorganism invasion is the innate immune system<sup>[44]</sup>. Unlike the

adaptive immunity, the response mounted by the innate immune system is relatively nonspecific, that mediated primarily by macrophages, dendritic cells (DCs), and granulocytes, basically functioning as phagocytes and APCs<sup>[45]</sup>.

The innate immune response depends on the recognition of the microbial-associated molecular patterns (MAMPs), through special cell receptors called pattern recognition receptors (PRRs)<sup>[46,47]</sup>. PRRs enable innate immune system to sense and recognize specific microbial compounds known as MAMPs<sup>[46]</sup>. PRRs comprise at least three distinct families: RA-inducible gene-I-like helicases, nucleotide oligomerization domain-like receptors (NLRs), and Toll-like receptors (TLRs)<sup>[48]</sup>.

The TLR family, best known and characterized in mammals, is composed of 13 receptors, which when activated cause activation of the immune system<sup>[49]</sup>. TLRs are able to recognize extracellular and endocytosed ligands<sup>[50]</sup>. Activation of TLR starts a cascade of pro-inflammatory reactions that leads to increased expression of specific cytokines, chemokines, and co-stimulatory molecules<sup>[51]</sup>. In type 1 diabetes, it has been demonstrated that TLRs, as the result of autoreactive processes directed against self antigens, may be priming an unwarranted adaptative immune response<sup>[52]</sup>. According to a study of Devaraj *et al.*<sup>[53]</sup> the monocytes attained from type 1 diabetic patients expressed TLR2 and TLR4 more than the control group. Furthermore shown in these patients the TLRs activity as well as the targets of the downstream TLR signaling including nuclear factor- $\kappa$ B (NF- $\kappa$ B), MyD88, and TIR-domain-containing adapter-inducing interferon (IFN)- $\beta$  were all respectively more expressed. So, TLR2 and TLR4 signaling may have significant role in development of type 1 diabetes<sup>[54]</sup>. However, TLR3 is not required for onset of autoimmune diabetes, while TLR9-deficient compared to TLR9 heterozygotes mice showed a significantly decreased incidence of diabetes<sup>[55]</sup>.

NLRs and C-type lectin receptors have not been reported to be directly related to autoimmunity. However, they may trigger autoimmune responses or initiate the adaptive immune system by autoimmune mechanisms<sup>[56]</sup>.

### Macrophage

The early studies indicated the role of macrophages in the pathogenesis of T1DM<sup>[57]</sup>. It has been reported that the islet infiltrates of young non-obese diabetic (NOD) mice contain macrophages and if the influx of these cells into the pancreas is inhibited, development of type 1 diabetes is prevented<sup>[58]</sup>. In addition, according to animal models macrophages produce proinflammatory cytokines such as tumor necrosis factor (TNF)- $\alpha$  and interleukin (IL)-1 $\beta$  which could be pathogenic for B cells<sup>[59,60]</sup>. Cytotoxic T cells are activated in the presence of macrophages, which subsequently destroy pancreatic  $\beta$ -cells<sup>[61]</sup>. Overall, the current evidence supports a pathogenic role for macrophages in initiation and development of T1DM.

### DCs

DCs, a group of diverse intrinsic effectors which have

two main actions relevant to T cell immune system controlling, including: Presentation of antigens to T cells and determining the nature of T cell response<sup>[62]</sup>. *In vivo*, as compared to healthy controls, DCs were located around the pancreatic islets in type 1 diabetic patients<sup>[63]</sup>. DCs present in the pancreatic islets of type 1 diabetic patient which suggests that these cells have a direct or indirect role in  $\beta$ -cell destruction<sup>[63]</sup>. In T1DM, DCs function as important APCs, DCs degrade the T cell response to antigen presentation<sup>[63]</sup>. Together, these studies support a diabetogenic role for DCs in the initiation steps of this disease.

### Natural killer cells

Natural killer (NK) cells can recognize and kill virus-infected cells through a number of different mechanisms; in addition NK cells have a critical role in immune regulation<sup>[64]</sup>. Data on NK cells in patients with T1DM are inconclusive<sup>[1]</sup>. Following stimulation by pro-inflammatory cytokines, NK cells generate a large quantity of cytokines such as IFN- $\gamma$ , TNF- $\alpha$ , and granulocyte macrophage-colony-stimulating factor (GM-CSF)<sup>[65]</sup>. According to animal models, NK cells can play a consequential role in the development of T1DM; however human studies accomplished in this field are rare. Evidence from animal model and man have shown that NK cells are potentially involved both in progression and protective of type 1 diabetes, thus suggesting a dual role for these cells in type 1 diabetes pathogenesis<sup>[66]</sup>. NK cells shown differently role in different stages of diseases to the disease pathogenesis<sup>[66]</sup>. These cells are the main source of IFN- $\gamma$ , and therefore, they regulate the intensity of the immune attack in diabetes and also the progression of insulinitis to diabetes<sup>[67]</sup>.

The NK receptors consist of two main families including NKG2A, for HLA E molecules, and the killer cell immunoglobulin-like receptors (KIR), for the recognition of HLA A, B, and C molecules<sup>[68]</sup>. Increased frequency of KIR gene haplotypes has been observed in patients with type 1 diabetes<sup>[68]</sup>.

In the blood of diabetic patients and in lymphoid tissues of NOD mice NK cell function has been impaired. Also, in type 1 diabetic patients a slight decrease of NKG2D expression has been observed. However animal studies suggested that NK cells activity was detected only in the early pre-diabetic infiltrates<sup>[1]</sup>. Most pancreatic NK cells of NOD mice became hypo-responsive during the later stages of diabetes development, as it is detected by lower cytokine secretion and a higher tendency for degranulation as a reaction toward antibodies which are distinct for receptor activation<sup>[69]</sup>.

According to the available evidence, NK cells can exhibit protective functions in  $\beta$ -cell autoimmunity conceivably by the down-regulation of T-cell lymphocytes and by the generation of IFN- $\gamma$ .

### Neutrophils

Neutrophils are a part of the immune system which do

not act specifically. These cells have a key role in the host immune system against different bacterial infections during the early host response to infection<sup>[1]</sup>. Neutrophils express many chemokine receptors, including CXCR1 and CXCR2, which respond to early chemokines released by macrophages. Neutrophils also express chemotactic receptors for complement, lipid mediators, and bacterial products<sup>[70,71]</sup>. So, neutrophils react to different chemo-attractants including lipid mediators, complement fragments and bacterial products<sup>[72,73]</sup>.

Many studies for the roles of neutrophils in the pathogenesis of diabetic complications have been carried out and ended with many controversies. According to previous studies, neutrophil dysfunction in chemotaxis, phagocytosis, killing bacteria and the release of superoxide in type 1 diabetic patients and animal models are not a cause but an effect of disease<sup>[74-77]</sup>.

## VITAMIN A AND INNATE IMMUNE SYSTEM

### Macrophage

The available evidence has introduced retinoids as important regulators of monocytic/macrophages function<sup>[78-81]</sup>. According to RA effect on monocytic/macrophages, it is shown that RA restrains the secretion of cytokines that promote the production of Th1-type cells and also it increases the secretion of cytokines that promote the production of Th2-type cells<sup>[82]</sup>. Macrophages secrete cytokines like TNF and nitric oxide (NO) under activation conditions<sup>[83]</sup>. RA affects the secretion of major cytokines generated by macrophages, including TNF- $\alpha$ , IL-1, IL-6, and IL-12<sup>[81]</sup>. A number of studies have shown that all-trans-RA extremely reduced the mRNA levels of TNF, regulates NO production, and increases IL-1 generation<sup>[82]</sup>.

Kim *et al.*<sup>[84]</sup> studied the impact of RA on a mouse model macrophage and its oblique effect on T cells. In their study they pretreated the macrophages with RA and precedingly activated them with lipopolysaccharides. In regard to the previous study, it was shown that RA inhibited the production of pro-inflammatory mediators (IL-12 production) by activating macrophages and the macrophages treated with RA when applied as antigen presenting cells (APCs) decreased the T-cell production of IFN- $\gamma$  and increase the generation of IL-4. Collectively RA signaling seems to set up a Th2-Treg non-inflammatory base<sup>[84,85]</sup>. Using RA-treated macrophages as APCs in co-cultures, result in IL-12 reduction and also T cell-derived IFN- $\gamma$  and IL-4 levels down-regulated and up-regulated, significantly<sup>[86]</sup>. Supplementation with vitamin A at 6500 IU/d for 6 mo in 6 patients with common variable immunodeficiency, who had low serum retinol concentrations, decreased the TNF- $\alpha$  level in comparison to onset levels<sup>[87]</sup>. The overall results show that supplementing with the preformed vitamin A may decrease the production of particular proinflammatory cytokines [monocyte-derived DCs (MoDCs), TNF- $\alpha$  and IL-6] by macrophages<sup>[8]</sup> (Figure

1).

### DCs

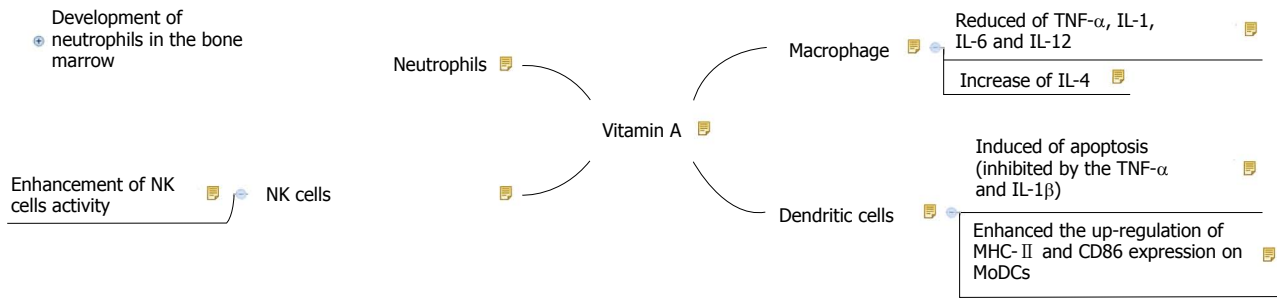
DCs, the primitive guardian cells which activate the development of adaptive immunity, can act as APCs and establish immune responses<sup>[88]</sup>. Therefore, RA's influence on this kind of cell could have a major role in initiating the adaptive immunity<sup>[79]</sup>. Apoptosis was induced by retinoids in immature MoDCs, this is inhibited by the secretion of cytokines like TNF- $\alpha$  and IL-1 $\beta$ . Also, retinoids enhanced the up-regulation of MHC-II and CD86 expression on MoDCs<sup>[79]</sup>. IL-4 and RA act synergistically on some populations of DCs and reduce the production of proinflammatory cytokines<sup>[89,90]</sup>. Furthermore RA could regulate the immunosuppressive properties of human tolerogenic DC and also mediate the transformation of B cells into B-regs<sup>[91]</sup> (Figure 1).

### NK cells

NK cells, part of the innate immune system, are critical in the first line of defense against tumors and viral infections<sup>[92]</sup>. These cells play an immune-regulatory role in antibody production and cell-mediated immunity through their production of various cytokines<sup>[93]</sup>. Previous investigations reported that vitamin A deficiency has a fundamental effect on NK cell lytic activity in young rodents<sup>[94]</sup>. Deficiency of vitamin A reduces the activity of NK cells and the ability of spleen cells to produce IFN after mitogen stimulation<sup>[95,96]</sup>. According to *in vitro* and *in vivo* studies, using physiologic or high concentrations of retinoids result in an enhancement of NK cell activity<sup>[97-99]</sup>. However, the mechanism for this stimulation is not fully clear<sup>[99]</sup>. A U-shaped relationship between vitamin A and NK cells has suggested which both low and high doses of vitamin A may have deleterious effects on NK cell hematopoiesis, differentiation or function<sup>[100]</sup>. In addition there is an interesting progressive relationship between the degree of vitamin A deficiency and the observed immune-suppression<sup>[101]</sup> (Figure 1).

### Neutrophils

The importance of the neutrophils is recognized in animals with neutropenia or a deficiency of any key neutrophils enzymes<sup>[102,103]</sup>. In these animals, mild infections can be life threatening<sup>[102,103]</sup>. The neutrophils differentiates requires the oxidized form of retinol, RA<sup>[104,105]</sup>. The development of neutrophils in the bone marrow is controlled by the genes that are modulated by RA receptors, and RA in cultures accelerates maturation of neutrophils<sup>[106,107]</sup>. According to previous studies, treatment with RA or vitamin A could restore the level of neutrophils and the capacity of superoxide-production in calves and rats significantly<sup>[108,109]</sup>. Vitamin A deficient rats had significantly higher numbers of hyper-segmented neutrophils (67%) relative to those in the control rats<sup>[103]</sup>. However the data on the relationship between vitamin A and neutrophils function in humans are sparse and inconclusive<sup>[8]</sup> (Figure 1).



**Figure 1** Schematic diagram for the pathways of vitamin A effects on innate immune system. NK: Natural killer; TNF: Tumor necrosis factors; IL: Interleukin; MoDCs: Monocyte-derived dendritic cells.

## TYPE 1 DIABETES AND ADAPTIVE IMMUNE RESPONSES

The adaptive immune system, which is the body's second defense system against pathogens, functions by its antigen-specific structure which distinguishes foreign molecules by their antigens which is mediated by the interaction between T cells and APCs. It generates long-term response by using immunological memory. T cells and T cell receptor are the essential section in the adaptative immune system<sup>[1]</sup>.

It is currently accepted that T cells play an important role in type 1 diabetes pathogenesis<sup>[110]</sup>. These cells are the most important players in the autoimmune attack of  $\beta$ -cells<sup>[110]</sup>. Anti-islet T cells, including both: CD4 and CD8 T cells have been observed in patients with T1DM<sup>[111]</sup>. It has been shown that transfer of anti-islet specific CD4 or CD8 T cells can lead to diabetes, as follows; insulinitis and diabetes can be adoptively transferred by T cells from diabetic mice into non-diabetic mice, whereas B cells are not needed<sup>[111-113]</sup>. In fact insulinitis seen in T1DM is induced by diabetogenic T cells that then recruit heterogeneous mixture of cells<sup>[114]</sup>.

CD8 T cells directly attack  $\beta$ -cells by MHC type 1 expressed on pancreatic  $\beta$ -cells, therefor in the absence of beta-2 microglobulin which reduces MHC type 1 or in the status of beta-restricted MHC type 1 deficiency, it is adequate to stop diabetes development and to avoid  $\beta$ -cell demolition in NOD mice<sup>[115,116]</sup>.

CD4 T cells are activated by  $\beta$ -cell APCs, and they mainly provide cytokines such as IL-21 to help both B cells and CD8 T cells, which is required for the development of T1DM in NOD mice. CD4 T cells secrete IFN- $\gamma$ , stimulating macrophages to release other cytokines, such as IL-1 $\beta$ , TNF- $\alpha$ , and free radicals, which are toxic to  $\beta$ -cells<sup>[44]</sup>.

Lymphocytes can kill  $\beta$ -cells directly through a cytotoxic process or by the secretion of proinflammatory cytokines, such as IL-1 $\beta$ , IFN- $\gamma$ ; they also release free radicals, which destructs the pancreatic  $\beta$ -cells. Cytokines induce the production of inducible NO synthase which results in NO production and NO synthesis influences the  $\beta$ -cell death<sup>[117]</sup>. Free radicals can induce, in turn, apoptosis and necrosis of  $\beta$ -cells<sup>[118]</sup>.

In the disease progression phase, both T and B lymphocytes can be activated against self antigens in

an islet lesion and trigger an immunological response that leads to the destruction of pancreatic  $\beta$ -cells<sup>[119,120]</sup> (Figure 2).

## VITAMIN A AND ADAPTIVE IMMUNE RESPONSES

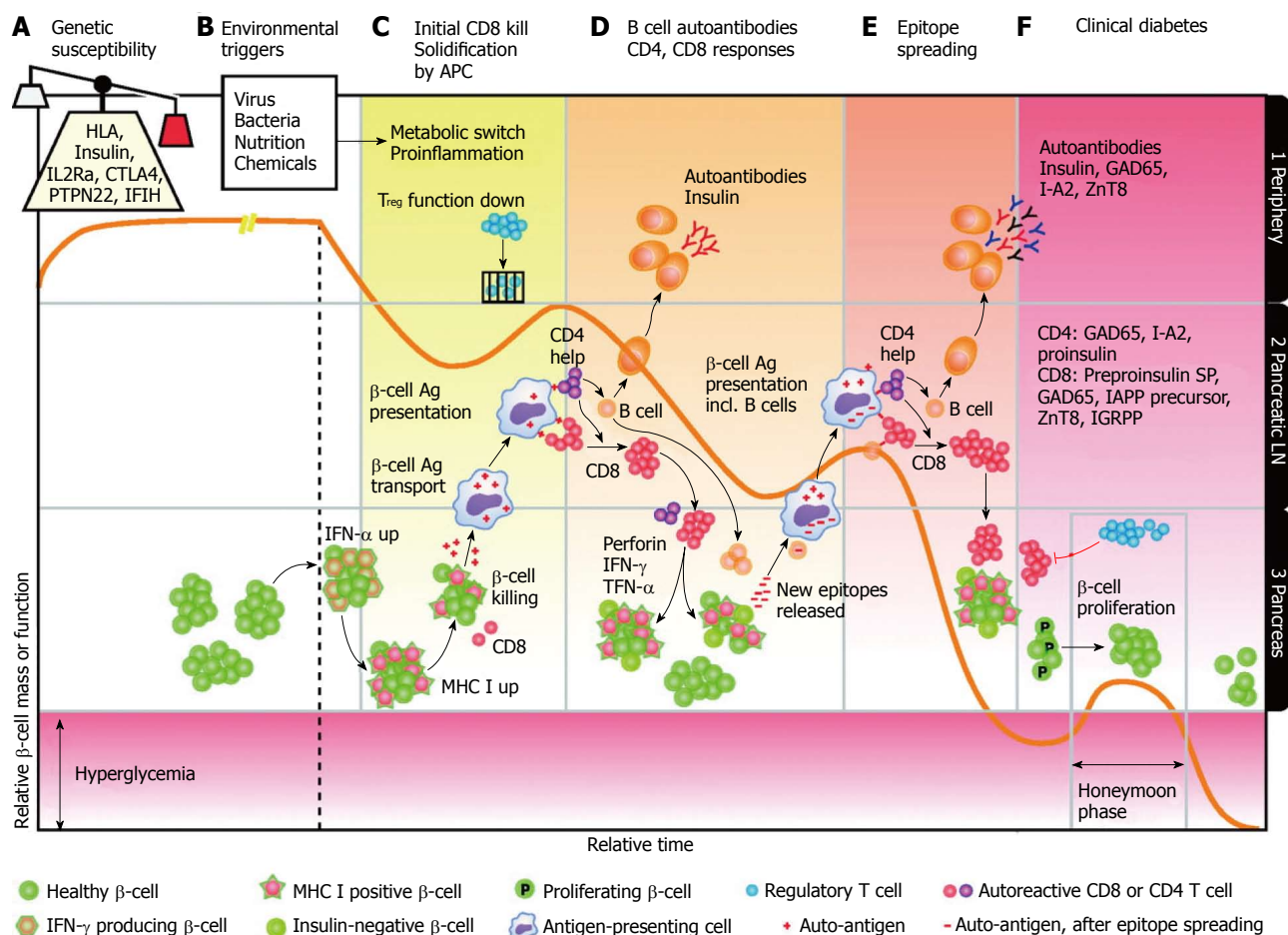
A study by Iwata *et al.*<sup>[121]</sup> for the first time described the role of RA in the biology of T cells. Recently evidenced T-cell immune-competence can be affected by vitamin A deficiency<sup>[8]</sup>.

Transforming growth factor (TGF)- $\beta$  which is a suppressor of Th1 and Th2 differentiation and the inducer of transforming T-cells to Tregs or to Th17, mediates RA in the process of formation, differentiation and inhibition or activation of Th1, Th2, Th17 and Treg lymphocytes<sup>[122]</sup>. The main impact of RA in lymphocyte differentiation by TGF- $\beta$  is the transformation of Th17<sup>[123,124]</sup>. RA has a two way affected on Th17 which on one hand it promotes its differentiation and on the other hand it downregulates it<sup>[125]</sup>. Th17 appears to be the example of customized immunity for special types of pathogens, but the abnormal Th17 responses could be involved in an exceeding number of autoimmune dysfunctions<sup>[126]</sup>. In some types of irregular immune responses, the defective form of RA *via* a genetic or an environmental mechanism intermediates the complex regulation of Th17<sup>[127]</sup>.

A growing number of evidence indicates that vitamin A is involved in the modulation of IL-10 production. IL-10, secreted by Th2-helper T cells, restricts the production of pro-inflammatory Th1-type cytokines, such as IFN- $\gamma$  and IL-2, in both T and NK cells. This is a major mechanism in reducing the inflammatory responses to some defects<sup>[128]</sup>.

A recent review demonstrated that in the status of vitamin A deficiency, Th1-cells mediate immune responses and when vitamin A is supplemented it induces Th2 immune responses<sup>[129]</sup>. Results from a number of studies that investigated the effect of vitamin A on infections that lead to one of the two immune responses, Th1 or a Th2 response, intimate that the immunological functions of vitamin A are specific for every pathogene and may involve other parts of the immune system other than Th1 or a Th2. Further studies are needed to examine the mechanism of vitamin A





**Figure 2 Immunology of type 1 diabetes**<sup>[115]</sup>. TNF: Tumor necrosis factors; IL: Interleukin; MoDCs: Monocyte-derived dendritic cells; APCs: Antigen presenting cells; IFN: Interferon; HLA: Human leukocyte antigen.

supplementation on differential of Th1/Th2 responses comparing to the baseline vitamin A status in humans and the specific pathogens that cause disorders<sup>[8]</sup>.

Overall, there is no exact evidence to state for a direct role of vitamin A supplementation on cytokine secretion or lymphocyte production. One principle reason for the wide range of results in studies is the specific immune response toward each pathogen which may affect the impact of vitamin A on T-cell function. Also, vitamin A may have momentary effects on intermediary factors of T-cell-dependent immunity which may not have been noticed in some population studies. Few randomized clinical trials have been accomplished on the topic of vitamin A supplementation on the proliferation or activation of B lymphocytes<sup>[8]</sup>.

## REVIEWS ON T1DM AND VITAMIN A

In the status of lack of balance between different subtypes of T-cells, autoimmune diseases occur<sup>[130]</sup>. For instance, IFN- $\gamma$ -producing CD4 or CD8T effectors (Teff) cells activation and expansion and/or reduction of the number or function of CD4T regulatory (Treg) cells, can result in autoimmune diseases<sup>[130-134]</sup>. Present evidence showed that both CD4<sup>+</sup> and CD8<sup>+</sup> Teff cells

are related in the initiation and further development of type 1 diabetes<sup>[135-137]</sup>. CD4<sup>+</sup> and CD8<sup>+</sup> Teff cells which are typically reactive with antigens on the  $\beta$ -cells in the pancreas can cause type 1 diabetes, an autoimmune disease<sup>[138]</sup>. Recent studies introduced IL-17-producing CD4 Th17 cells as a new generation of Teff cells that trigger potent inflammatory responses resulting in autoimmune disorders<sup>[139]</sup>. These results suggest that the establishment of effective *in vivo* immune-tolerance can be considered as practical strategy to treat autoimmune diseases such as type 1 diabetes. However this approach requires simultaneous targeting of more than one T-cell population subset. Therefore, immune tolerance induced by clinically relevant agents or methods affecting various T-cell subtypes could demonstrate an effective way for treating human autoimmune disorders<sup>[140]</sup>.

Vitamin A and its derivatives are potent immune tolerance agents by its ability to transform Th1 to Th2 lymphocytes<sup>[138,140]</sup>. Vitamin A regulates the adaptive and innate immune responses by different mechanisms for example, its high-level can diminish development of Th1 and promote development of Th2 responses<sup>[141]</sup>. Vitamin A supplementation results in a decrease in serum pro-inflammatory cytokines, such as TNF- $\alpha$  and IFN- $\gamma$ , and an increase in the immunosuppressive cytokine

IL-10<sup>[87,142]</sup>. Such immune modulation by vitamin A could decrease the development risk of autoimmune diseases<sup>[143]</sup>. Present evidence reported high level of dietary vitamin A may have major effects on down regulating inflammatory immune cells and reducing the damage caused by oxidation in the islets that contribute to dysfunction of  $\beta$  cells. An animal study conducted by Zunino *et al.*<sup>[138]</sup> showed that intervention with a diet rich in vitamin A inhibited the development of type I diabetes in mice by reducing or delaying of the infiltration of immune cells in to the islets.

Furthermore vitamin A plays a role in the release of insulin and glucagon hormones<sup>[12]</sup>, and therefore has profound effects throughout the body in the regulation of glucose homeostasis<sup>[12,13]</sup>. Vitamin A in active form has an important role in the secretion and release of insulin in the langerhans islets cells. The presence of RA binding proteins in pancreatic islet cells could probably explain the significance of vitamin A for optimal islet function<sup>[12]</sup>. Furthermore RA may be involved in regulation of the hormones released by the islet cells<sup>[12]</sup>. Deficient islet cells were defective in hormone release when exposed to graded levels of glucose<sup>[12,13]</sup>. Vitamin A deficiency results in change in pancreatic tissue quality, which could conceivably increase digestion with the collagenase<sup>[144]</sup>.

In an animal study, NOD mice were divided into 3 groups and treated with 250 IU of vitamin A per gram of their daily food or treated with 1% freeze-dried grape powder in their diet or a control diet for 7 mo. After 7 mo, in the control group 71% of the mice had a blood sugar level more than 13.9 mmol/L (full-blown T1DM) whereas only 25% of the mice in the vitamin A group and 33% of the grape powder group reached the above blood sugar level. Furthermore TNF- $\alpha$ , an inflammatory marker in T1DM patients, in the vitamin A and grape powder groups was respectively lower compared to the control group<sup>[145]</sup>. These results suggested that polyphenols or vitamin A in the diet protect beta-cell islets against autoimmune inflammatory attacks and have the potency to decrease the formation of autoimmune diseases such as type 1 diabetes<sup>[146]</sup>.

In addition it has been showed that all-trans retinoic acid (ATRA), a potent derivative of vitamin A treatment restricted both CD4<sup>+</sup> and CD8<sup>+</sup> IFN- $\gamma$  producing cells without affecting CD4<sup>+</sup> IL-17-producing cells. ATRA treatment also affects the function and activation status of CD8<sup>+</sup> T-cells<sup>[140]</sup>. Macrophages generate less TNF- $\alpha$  which in return reduces the production of chemokines which promote the recruitment of immune cells in to the islets including IP-10, RANTES, and MIP-1b, and also intracellular adhesion molecule-1<sup>[147,148]</sup>. According to histological studies, non diabetic animal treated with ATRA did not have insulinitis, indicating that ATRA may have also inhibited T-cells trafficking to and infiltration in to islets, thus preventing diabetes. Furthermore, recently *in vitro* and *in vivo* trials indicated that ATRA treatment may result in upregulation of Foxp3<sup>+</sup> Treg cells and reduction of Th1 and Th17 cell differentiation<sup>[125,141,149]</sup>. Protective effects of ATRA are impairing in the status

of inadequacy of donors Foxp3<sup>+</sup> CD4<sup>+</sup> Treg cells<sup>[140]</sup>. Overall, these evidences support the idea that vitamin A and its derivatives exerted its autoimmune-protective effect, at least in part, by inhibiting both CD4<sup>+</sup> and CD8<sup>+</sup> IFN- $\gamma$ -producing Teff cells with no effects on IL-17-producing Teff cells, and inducing the production of Treg cells. However despite the fact that ATRA treatment inhibited the *in vitro* differentiation of Th17 cells did not alter the Th17 cell population<sup>[125,141,149,150]</sup>. Although Th17 cells are important players in pathogenesis of some autoimmune diseases including experimental autoimmune encephalitis and autoimmune arthritis, its function in type 1 diabetes is not yet discovered<sup>[151-153]</sup>. Expression of granzyme B was suppressed by ATRA. In addition ATRA efficiently inhibits infiltration of T-cells into islets, and precluded the progression of insulinitis and diabetes. A study conducted by Van *et al.*<sup>[140]</sup> showed that defect less islets or pre-insulinitis were detected in ATRA-treated mice, even after 17 wk of the cell transfer while the control group developed severe destructive insulinitis at 2 wk after cell transfer with CD4 CD25.

## CONCLUSION

This review reported that both vitamin A and ATRA effectively induced immune tolerance that inhibited islet inflammation and progression to diabetes. In this review, as fully mentioned previously we showed that ATRA treatment had a dual effect, the inhibition of Teff cells and inducing Treg cell proliferation in therapeutic of type 1 diabetes. Nonetheless, the protective effect of ATRA is inhibited when CD4CD25 T-cells, thus a majority of Foxp3 Treg, are drawn down in donor splenocytes. In prediabetic NOD mice with initiated insulinitis, ATRA treatment can inhibit the development of T1DM. Nevertheless, the mechanisms demonstrating the role of vitamin A or ATRA treatment in inducing immune tolerance and prevention of autoimmune diseases is not yet clear<sup>[138,142,154-156]</sup>. So, to further validate and establish the potential of using ATRA for therapy, further studies are needed to evaluate its relative contribution in modulating type 1 diabetes and to show the mechanisms by which vitamin A and ATRA may inhibit the development of autoimmune disorders. Overall, it seems that the use of vitamin A and ATRA *via* induction of immune tolerance provides an effective method in inhibiting type 1 diabetes.

## ACKNOWLEDGMENTS

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## REFERENCES

- 1 Szablewski L. Role of immune system in type 1 diabetes mellitus pathogenesis. *Int Immunopharmacol* 2014; **22**: 182-191 [PMID: 24993340 DOI: 10.1016/j.intimp.2014.06.033]
- 2 Atkinson MA, Eisenbarth GS. Type 1 diabetes: new perspectives on disease pathogenesis and treatment. *Lancet* 2001; **358**: 221-229 [PMID: 11476858 DOI: 10.1016/s0140-6736(01)05415-0]

- 3 **Patterson CC**, Dahlquist GG, Gyürüs E, Green A, Soltész G. Incidence trends for childhood type 1 diabetes in Europe during 1989-2003 and predicted new cases 2005-20: a multicentre prospective registration study. *Lancet* 2009; **373**: 2027-2033 [PMID: 19481249 DOI: 10.1016/S0140-6736(09)60568-7]
- 4 **Laing SP**, Swerdlow AJ, Slater SD, Botha JL, Burden AC, Waugh NR, Smith AW, Hill RD, Bingley PJ, Patterson CC, Qiao Z, Keen H. The British Diabetic Association Cohort Study, II: cause-specific mortality in patients with insulin-treated diabetes mellitus. *Diabet Med* 1999; **16**: 466-471 [PMID: 10391393 DOI: 10.1046/j.1464-5491.1999.00076.x]
- 5 **Federation ID**. IdF diabetes atlas. Brussels: International Diabetes Federation, 2013
- 6 **Hassan GA**, Sliem HA, Ellethy AT, Salama Mel-S. Role of immune system modulation in prevention of type 1 diabetes mellitus. *Indian J Endocrinol Metab* 2012; **16**: 904-909 [PMID: 23226634 DOI: 10.4103/2230-8210.102989]
- 7 **Bougnères PF**, Landais P, Boisson C, Carel JC, Frament N, Boitard C, Chaussain JL, Bach JF. Limited duration of remission of insulin dependency in children with recent overt type I diabetes treated with low-dose cyclosporin. *Diabetes* 1990; **39**: 1264-1272 [PMID: 2210078 DOI: 10.2337/diab.39.10.1264]
- 8 **Villamor E**, Fawzi WW. Effects of vitamin a supplementation on immune responses and correlation with clinical outcomes. *Clin Microbiol Rev* 2005; **18**: 446-464 [PMID: 16020684 DOI: 10.1128/CMR.18.3.446-464.2005]
- 9 **Green HN**, Mellanby E. Vitamin A as an anti-infective agent. *Br Med J* 1928; **2**: 691-696 [PMID: 20774205 DOI: 10.1136/bmj.2.3537.691]
- 10 **Green H**, Mellanby E. Carotene and vitamin A: The anti-infective action of carotene. *Brit J Exper Path* 1930; **11**: 81-89
- 11 **Ross AC**. Vitamin A and retinoic acid in T cell-related immunity. *Am J Clin Nutr* 2012; **96**: 1166S-1172S [PMID: 23053562 DOI: 10.3945/ajcn.112.034637]
- 12 **Berdanier C**. Vitamin A needs in diabetes mellitus. *Sight and Life Newsletter* 2003; **1**: 3-15
- 13 **Berdanier CD**, Everts HB, Hermoyian C, Mathews CE. Role of vitamin A in mitochondrial gene expression. *Diabetes Res Clin Pract* 2001; **54** Suppl 2: S11-S27 [PMID: 11733105 DOI: 10.1016/S0168-8227(01)00331-X]
- 14 **Chertow BS**, Blaner WS, Baranetsky NG, Sivitz WI, Cordle MB, Thompson D, Meda P. Effects of vitamin A deficiency and repletion on rat insulin secretion in vivo and in vitro from isolated islets. *J Clin Invest* 1987; **79**: 163-169 [PMID: 3025258 DOI: 10.1172/JCI112778]
- 15 **Driscoll HK**, Adkins CD, Chertow TE, Cordle MB, Matthews KA, Chertow BS. Vitamin A stimulation of insulin secretion: effects on transglutaminase mRNA and activity using rat islets and insulin-secreting cells. *Pancreas* 1997; **15**: 69-77 [PMID: 9211495 DOI: 10.1097/00006676-199707000-00010]
- 16 **Olmedilla B**, Granado F, Gil-Martinez E, Blanco I, Rojas-Hidalgo E. Reference values for retinol, tocopherol, and main carotenoids in serum of control and insulin-dependent diabetic Spanish subjects. *Clin Chem* 1997; **43**: 1066-1071 [PMID: 9191562]
- 17 **Granado F**, Olmedilla B, Botella F, Simal A, Blanco I. Retinol and alpha-tocopherol in serum of type 1 diabetic patients with intensive insulin therapy: a long term follow-up study. *Nutrition* 2003; **19**: 128-132 [PMID: 12591543 DOI: 10.1016/S0899-9007(02)00908-5]
- 18 **Kobbah AM**, Hellsing K, Tuvemo T. Early changes of some serum proteins and metals in diabetic children. *Acta Paediatr Scand* 1988; **77**: 734-740 [PMID: 3059755 DOI: 10.1111/j.1651-2227.1988.tb10739.x]
- 19 **Basu TK**, Tze WJ, Leichter J. Serum vitamin A and retinol-binding protein in patients with insulin-dependent diabetes mellitus. *Am J Clin Nutr* 1989; **50**: 329-331 [PMID: 2756919]
- 20 **Nakamura A**, Osonoi T, Terauchi Y. Relationship between urinary sodium excretion and pioglitazone-induced edema. *J Diabetes Investig* 2010; **1**: 208-211 [PMID: 24843434 DOI: 10.1111/j.2040-1124.2010.00046.x]
- 21 **Jain SK**, McVie R, Duett J, Herbst JJ. The effect of glycemic control on plasma prealbumin levels in type-1 diabetic children. *Horm Metab Res* 1993; **25**: 102-104 [PMID: 8458604 DOI: 10.1055/s-2007-1002052]
- 22 **Granado F**, Olmedilla B, Gil-Martínez E, Blanco I, Millán I, Rojas-Hidalgo E. Carotenoids, retinol and tocopherols in patients with insulin-dependent diabetes mellitus and their immediate relatives. *Clin Sci (Lond)* 1998; **94**: 189-195 [PMID: 9536928 DOI: 10.1042/cs0940189]
- 23 **Gottesman ME**, Quadro L, Blaner WS. Studies of vitamin A metabolism in mouse model systems. *Bioessays* 2001; **23**: 409-419 [PMID: 11340622 DOI: 10.1002/bies.1059]
- 24 **Niederreither K**, Dollé P. Retinoic acid in development: towards an integrated view. *Nat Rev Genet* 2008; **9**: 541-553 [PMID: 18542081 DOI: 10.1038/nrg2340]
- 25 **Blomhoff R**, Blomhoff HK. Overview of retinoid metabolism and function. *J Neurobiol* 2006; **66**: 606-630 [PMID: 16688755 DOI: 10.1002/neu.20242]
- 26 **Napoli JL**. Physiological insights into all-trans-retinoic acid biosynthesis. *Biochim Biophys Acta* 2012; **1821**: 152-167 [PMID: 21621639]
- 27 **Vogel S**, Gamble M, Blaner W. Biosynthesis, absorption, metabolism and transport of retinoids. In: Retinoids, Section A, editors. Berlin Heidelberg: Springer, 1999: 31-95 [DOI: 10.1007/978-3-642-58483-1\_2]
- 28 **O'Byrne SM**, Blaner WS. Retinol and retinyl esters: biochemistry and physiology. *J Lipid Res* 2013; **54**: 1731-1743 [PMID: 23625372 DOI: 10.1194/jlr.R037648]
- 29 **Vieira AV**, Schneider WJ, Vieira PM. Retinoids: transport, metabolism, and mechanisms of action. *J Endocrinol* 1995; **146**: 201-207 [PMID: 7561630 DOI: 10.1677/joe.0.1460201]
- 30 **Quadro L**, Hamberger L, Colantuoni V, Gottesman ME, Blaner WS. Understanding the physiological role of retinol-binding protein in vitamin A metabolism using transgenic and knockout mouse models. *Mol Aspects Med* 2003; **24**: 421-430 [PMID: 14585313]
- 31 **Sporn M**, Roberts A, Goodman D. The retinoids: Biology, chemistry and medicine. New York: Raven Press, 1994: 351
- 32 **Duester G**. Families of retinoid dehydrogenases regulating vitamin A function: production of visual pigment and retinoic acid. *Eur J Biochem* 2000; **267**: 4315-4324 [PMID: 10880953 DOI: 10.1046/j.1432-1327.2000.01497.x]
- 33 **Dowling JE**, Wald G. The biological function of vitamin A acid. *Proc Natl Acad Sci USA* 1960; **46**: 587-608 [PMID: 16590647 DOI: 10.1073/pnas.46.5.587]
- 34 **Giguere V**, Ong ES, Segui P, Evans RM. Identification of a receptor for the morphogen retinoic acid. *Nature* 1987; **330**: 624-629 [PMID: 2825036 DOI: 10.1038/330624a0]
- 35 **Mangelsdorf DJ**, Ong ES, Dyck JA, Evans RM. Nuclear receptor that identifies a novel retinoic acid response pathway. *Nature* 1990; **345**: 224-229 [PMID: 2159111 DOI: 10.1038/345224a0]
- 36 **Pillay K**, Coutoudis A, Agadzi-Naqvi AK, Kuhn L, Coovadia HM, Janoff EN. Secretory leukocyte protease inhibitor in vaginal fluids and perinatal human immunodeficiency virus type 1 transmission. *J Infect Dis* 2001; **183**: 653-656 [PMID: 11170993 DOI: 10.1086/318535]
- 37 **Simmons KM**, Michels AW. Type 1 diabetes: A predictable disease. *World J Diabetes* 2015; **6**: 380-390 [PMID: 25897349 DOI: 10.4239/wjd.v6.i3.380]
- 38 **Harrison LC**, Honeyman MC, DeAizpurua HJ, Schmidli RS, Colman PG, Tait BD, Cram DS. Inverse relation between humoral and cellular immunity to glutamic acid decarboxylase in subjects at risk of insulin-dependent diabetes. *Lancet* 1993; **341**: 1365-1369 [PMID: 8098789 DOI: 10.1016/0140-6736(93)90940-I]
- 39 **Kaufman DL**, Clare-Salzler M, Tian J, Forsthuber T, Ting GS, Robinson P, Atkinson MA, Sercarz EE, Tobin AJ, Lehmann PV. Spontaneous loss of T-cell tolerance to glutamic acid decarboxylase in murine insulin-dependent diabetes. *Nature* 1993; **366**: 69-72 [PMID: 7694152 DOI: 10.1038/366069a0]
- 40 **Orban T**, Sosenko JM, Cuthbertson D, Krischer JP, Skyler JS, Jackson R, Yu L, Palmer JP, Schatz D, Eisenbarth G. Pancreatic



- islet autoantibodies as predictors of type 1 diabetes in the Diabetes Prevention Trial-Type 1. *Diabetes Care* 2009; **32**: 2269-2274 [PMID: 19741189 DOI: 10.2337/dc09-0934]
- 41 **Exton JH**. Mechanisms of hormonal regulation of hepatic glucose metabolism. *Diabetes Metab Rev* 1987; **3**: 163-183 [PMID: 3032541 DOI: 10.1002/dmr.5610030108]
  - 42 **Haller MJ**, Atkinson MA, Schatz D. Type 1 diabetes mellitus: etiology, presentation, and management. *Pediatr Clin North Am* 2005; **52**: 1553-1578 [PMID: 16301083 DOI: 10.1016/j.pcl.2005.07.006]
  - 43 **Michels AW**. Targeting the trimolecular complex. *Clin Immunol* 2013; **149**: 339-344 [PMID: 23537861 DOI: 10.1016/j.clim.2013.02.020]
  - 44 **Pietropaolo M**, Surhig JM, Nelson PW, Eisenbarth GS. Primer: immunity and autoimmunity. *Diabetes* 2008; **57**: 2872-2882 [PMID: 18971434 DOI: 10.2337/db07-1691]
  - 45 **Iwasaki A**, Medzhitov R. Toll-like receptor control of the adaptive immune responses. *Nat Immunol* 2004; **5**: 987-995 [PMID: 15454922 DOI: 10.1038/ni1112]
  - 46 **Janeway CA**, Medzhitov R. Innate immune recognition. *Annu Rev Immunol* 2002; **20**: 197-216 [PMID: 11861602 DOI: 10.1146/annurev.immunol.20.083001.084359]
  - 47 **Elia PP**, Tolentino YF, Bernardazzi C, de Souza HS. The role of innate immunity receptors in the pathogenesis of inflammatory bowel disease. *Mediators Inflamm* 2015; **2015**: 936193 [PMID: 25821356]
  - 48 **Wells JM**, Rossi O, Meijerink M, van Baarlen P. Epithelial crosstalk at the microbiota-mucosal interface. *Proc Natl Acad Sci USA* 2011; **108** Suppl 1: 4607-4614 [PMID: 20826446 DOI: 10.1073/pnas.1000092107]
  - 49 **Akira S**, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. *Cell* 2006; **124**: 783-801 [PMID: 16497588 DOI: 10.1016/j.cell.2006.02.015]
  - 50 **Hornef MW**, Normark BH, Vandewalle A, Normark S. Intracellular recognition of lipopolysaccharide by toll-like receptor 4 in intestinal epithelial cells. *J Exp Med* 2003; **198**: 1225-1235 [PMID: 14568981]
  - 51 **Takeda K**, Akira S. Toll-like receptors. *Curr Protoc Immunol* 2015; **109**: 14.12.1-14.12.10 [PMID: 25845562 DOI: 10.1084/jem.20022194]
  - 52 **Martin DA**, Elkon KB. Autoantibodies make a U-turn: the toll hypothesis for autoantibody specificity. *J Exp Med* 2005; **202**: 1465-1469 [PMID: 16330812 DOI: 10.1084/jem.20052228]
  - 53 **Devaraj S**, Dasu MR, Rockwood J, Winter W, Griffen SC, Jialal I. Increased toll-like receptor (TLR) 2 and TLR4 expression in monocytes from patients with type 1 diabetes: further evidence of a proinflammatory state. *J Clin Endocrinol Metab* 2008; **93**: 578-583 [PMID: 18029454 DOI: 10.1210/jc.2007-2185]
  - 54 **Pino SC**, Kruger AJ, Bortell R. The role of innate immune pathways in type 1 diabetes pathogenesis. *Curr Opin Endocrinol Diabetes Obes* 2010; **17**: 126-130 [PMID: 20125005 DOI: 10.1097/MED.0b013e3283372819]
  - 55 **Wong FS**, Hu C, Zhang L, Du W, Alexopoulou L, Flavell RA, Wen L. The role of Toll-like receptors 3 and 9 in the development of autoimmune diabetes in NOD mice. *Ann N Y Acad Sci* 2008; **1150**: 146-148 [PMID: 19120284 DOI: 10.1196/annals.1447.039]
  - 56 **Beyan H**, Buckley LR, Yousaf N, Londei M, Leslie RD. A role for innate immunity in type 1 diabetes? *Diabetes Metab Res Rev* 2003; **19**: 89-100 [PMID: 12673777 DOI: 10.1002/dmrr.341]
  - 57 **Gordon S**. Alternative activation of macrophages. *Nat Rev Immunol* 2003; **3**: 23-35 [PMID: 12511873 DOI: 10.1038/nri978]
  - 58 **Hutchings PR**, Cooke A. The transfer of autoimmune diabetes in NOD mice can be inhibited or accelerated by distinct cell populations present in normal splenocytes taken from young males. *J Autoimmun* 1990; **3**: 175-185 [PMID: 1971173 DOI: 10.1016/0896-8411(90)90139-J]
  - 59 **Arnush M**, Scarim AL, Heitmeier MR, Kelly CB, Corbett JA. Potential role of resident islet macrophage activation in the initiation of autoimmune diabetes. *J Immunol* 1998; **160**: 2684-2691 [PMID: 9510167]
  - 60 **Dahlén E**, Dawe K, Ohlsson L, Hedlund G. Dendritic cells and macrophages are the first and major producers of TNF-alpha in pancreatic islets in the nonobese diabetic mouse. *J Immunol* 1998; **160**: 3585-3593 [PMID: 9531322]
  - 61 **Martinez FO**, Helming L, Gordon S. Alternative activation of macrophages: an immunologic functional perspective. *Annu Rev Immunol* 2009; **27**: 451-483 [PMID: 19105661 DOI: 10.1146/annurev.immunol.021908.132532]
  - 62 **Tisch R**, Wang B. Role of plasmacytoid dendritic cells in type 1 diabetes: friend or foe? *Diabetes* 2009; **58**: 12-13 [PMID: 19114722 DOI: 10.2337/db08-1341]
  - 63 **Summers KL**, Behme MT, Mahon JL, Singh B. Characterization of dendritic cells in humans with type 1 diabetes. *Ann N Y Acad Sci* 2003; **1005**: 226-229 [PMID: 14679065 DOI: 10.1196/annals.1288.032]
  - 64 **Jost S**, Altfield M. Control of human viral infections by natural killer cells. *Annu Rev Immunol* 2013; **31**: 163-194 [PMID: 23298212 DOI: 10.1146/annurev-immunol-032712-100001]
  - 65 **Fauriat C**, Long EO, Ljunggren HG, Bryceson YT. Regulation of human NK-cell cytokine and chemokine production by target cell recognition. *Blood* 2010; **115**: 2167-2176 [PMID: 19965656 DOI: 10.1182/blood-2009-08-238469]
  - 66 **Dotta F**, Fondelli C, Falorni A. Can NK cells be a therapeutic target in human type 1 diabetes? *Eur J Immunol* 2008; **38**: 2961-2963 [PMID: 18979518 DOI: 10.1002/eji.200838851]
  - 67 **Rodacki M**, Svoren B, Butty V, Besse W, Laffel L, Benoist C, Mathis D. Altered natural killer cells in type 1 diabetic patients. *Diabetes* 2007; **56**: 177-185 [PMID: 17192480 DOI: 10.2337/db06-0493]
  - 68 **Rodacki M**, Milech A, de Oliveira JE. NK cells and type 1 diabetes. *Clin Dev Immunol* 2006; **13**: 101-107 [PMID: 17162353 DOI: 10.1080/17402520600877182]
  - 69 **Shi FD**, Ljunggren HG, La Cava A, Van Kaer L. Organ-specific features of natural killer cells. *Nat Rev Immunol* 2011; **11**: 658-671 [PMID: 21941294 DOI: 10.1038/nri3065]
  - 70 **Ariel A**, Fredman G, Sun YP, Kantarci A, Van Dyke TE, Luster AD, Serhan CN. Apoptotic neutrophils and T cells sequester chemokines during immune response resolution through modulation of CCR5 expression. *Nat Immunol* 2006; **7**: 1209-1216 [PMID: 17013391 DOI: 10.1038/ni1392]
  - 71 **Proebstl D**, Voisin MB, Woodfin A, Whiteford J, D'Acquisto F, Jones GE, Rowe D, Nourshargh S. Pericytes support neutrophil subendothelial cell crawling and breaching of venular walls in vivo. *J Exp Med* 2012; **209**: 1219-1234 [PMID: 22615129 DOI: 10.1084/jem.20111622]
  - 72 **McDonald B**, Pittman K, Menezes GB, Hirota SA, Slaba I, Waterhouse CC, Beck PL, Muruve DA, Kubas P. Intravascular danger signals guide neutrophils to sites of sterile inflammation. *Science* 2010; **330**: 362-366 [PMID: 20947763 DOI: 10.1126/science.1195491]
  - 73 **Sokol CL**, Luster AD. The chemokine system in innate immunity. *Cold Spring Harb Perspect Biol* 2015; **7**: pii: a016303 [PMID: 25635046 DOI: 10.1101/cshperspect.a016303]
  - 74 **Nabi AH**, Islam LN, Rahman MM, Biswas KB. Polymorphonuclear neutrophil dysfunctions in streptozotocin-induced type 1 diabetic rats. *J Biochem Mol Biol* 2005; **38**: 661-667 [PMID: 16336780 DOI: 10.5483/BMBRep.2005.38.6.661]
  - 75 **Shetty N**, Thomas B, Ramesh A. Comparison of neutrophil functions in diabetic and healthy subjects with chronic generalized periodontitis. *J Indian Soc Periodontol* 2008; **12**: 41-44 [PMID: 20142943 DOI: 10.4103/0972-124X.44089]
  - 76 **Hanses F**, Park S, Rich J, Lee JC. Reduced neutrophil apoptosis in diabetic mice during staphylococcal infection leads to prolonged Tnfa production and reduced neutrophil clearance. *PLoS One* 2011; **6**: e23633 [PMID: 21912601 DOI: 10.1371/journal.pone.0023633]
  - 77 **Alba-Loureiro TC**, Hirabara SM, Mendonça JR, Curi R, Pithon-Curi TC. Diabetes causes marked changes in function and metabolism of rat neutrophils. *J Endocrinol* 2006; **188**: 295-303 [PMID: 16461555 DOI: 10.1677/joe.1.06438]
  - 78 **Breitman TR**, Selonick SE, Collins SJ. Induction of differentiation of the human promyelocytic leukemia cell line (HL-60) by retinoic acid. *Proc Natl Acad Sci USA* 1980; **77**: 2936-2940 [PMID: 7111111]



- 6930676 DOI: 10.1073/pnas.77.5.2936]
- 79 **Geissmann F**, Revy P, Brousse N, Lepelletier Y, Folli C, Durandy A, Chambon P, Dy M. Retinoids regulate survival and antigen presentation by immature dendritic cells. *J Exp Med* 2003; **198**: 623-634 [PMID: 12925678 DOI: 10.1084/jem.20030390]
  - 80 **Jiang YJ**, Xu TR, Lu B, Mymyn D, Kroeger EA, Dembinski T, Yang X, Hatch GM, Choy PC. Cyclooxygenase expression is elevated in retinoic acid-differentiated U937 cells. *Biochim Biophys Acta* 2003; **1633**: 51-60 [PMID: 12842195 DOI: 10.1016/S1388-1981(03)00072-6]
  - 81 **Mohty M**, Morbelli S, Isnardon D, Sainty D, Arnoulet C, Gaugler B, Olive D. All-trans retinoic acid skews monocyte differentiation into interleukin-12-secreting dendritic-like cells. *Br J Haematol* 2003; **122**: 829-836 [PMID: 12930397 DOI: 10.1046/j.1365-2141.2003.04489.x]
  - 82 **Mehta K**, McQueen T, Tucker S, Pandita R, Aggarwal BB. Inhibition by all-trans-retinoic acid of tumor necrosis factor and nitric oxide production by peritoneal macrophages. *J Leukoc Biol* 1994; **55**: 336-342 [PMID: 8120450]
  - 83 **Sherry B**, Cerami A. Cachectin/tumor necrosis factor exerts endocrine, paracrine, and autocrine control of inflammatory responses. *J Cell Biol* 1988; **107**: 1269-1277 [PMID: 3049617 DOI: 10.1083/jcb.107.4.1269]
  - 84 **Kim BH**, Kang KS, Lee YS. Effect of retinoids on LPS-induced COX-2 expression and COX-2 associated PGE(2) release from mouse peritoneal macrophages and TNF-alpha release from rat peripheral blood mononuclear cells. *Toxicol Lett* 2004; **150**: 191-201 [PMID: 15093674 DOI: 10.1016/j.toxlet.2004.01.010]
  - 85 **Na SY**, Kang BY, Chung SW, Han SJ, Ma X, Trinchieri G, Im SY, Lee JW, Kim TS. Retinoids inhibit interleukin-12 production in macrophages through physical associations of retinoid X receptor and NFkappaB. *J Biol Chem* 1999; **274**: 7674-7680 [PMID: 10075655 DOI: 10.1074/jbc.274.12.7674]
  - 86 **Kang SG**, Lim HW, Andrisani OM, Broxmeyer HE, Kim CH. Vitamin A metabolites induce gut-homing FoxP3+ regulatory T cells. *J Immunol* 2007; **179**: 3724-3733 [PMID: 17785809 DOI: 10.4049/jimmunol.179.6.3724]
  - 87 **Aukrust P**, Müller F, Ueland T, Svandal AM, Berge RK, Frøland SS. Decreased vitamin A levels in common variable immunodeficiency: vitamin A supplementation in vivo enhances immunoglobulin production and downregulates inflammatory responses. *Eur J Clin Invest* 2000; **30**: 252-259 [PMID: 10692003 DOI: 10.1046/j.1365-2362.2000.00619.x]
  - 88 **Banchereau J**, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, Pulendran B, Palucka K. Immunobiology of dendritic cells. *Annu Rev Immunol* 2000; **18**: 767-811 [PMID: 10837075 DOI: 10.1146/annurev.immunol.18.1.767]
  - 89 **Klebanoff CA**, Spencer SP, Torabi-Parizi P, Grainger JR, Roychoudhuri R, Ji Y, Sukumar M, Muranski P, Scott CD, Hall JA, Ferreyra GA, Leonardi AJ, Borman ZA, Wang J, Palmer DC, Wilhelm C, Cai R, Sun J, Napoli JL, Danner RL, Gattinoni L, Belkaid Y, Restifo NP. Retinoic acid controls the homeostasis of pre-cDC-derived splenic and intestinal dendritic cells. *J Exp Med* 2013; **210**: 1961-1976 [PMID: 23999499 DOI: 10.1084/jem.20122508]
  - 90 **Zhu B**, Buttrick T, Bassil R, Zhu C, Olah M, Wu C, Xiao S, Orent W, Elyaman W, Khoury SJ. IL-4 and retinoic acid synergistically induce regulatory dendritic cells expressing Aldh1a2. *J Immunol* 2013; **191**: 3139-3151 [PMID: 23960232 DOI: 10.4049/jimmunol.1300329]
  - 91 **Di Caro V**, Phillips B, Engman C, Harnaha J, Trucco M, Giannoukakis N. Retinoic acid-producing, ex-vivo-generated human tolerogenic dendritic cells induce the proliferation of immunosuppressive B lymphocytes. *Clin Exp Immunol* 2013; **174**: 302-317 [PMID: 23865694 DOI: 10.1111/cei.12177]
  - 92 **Zhao Z**, Murasko DM, Ross AC. The role of vitamin A in natural killer cell cytotoxicity, number and activation in the rat. *Nat Immun* 1994; **13**: 29-41 [PMID: 8111191]
  - 93 **Kos FJ**. Regulation of adaptive immunity by natural killer cells. *Immunol Res* 1998; **17**: 303-312 [PMID: 9638474 DOI: 10.1007/BF02786453]
  - 94 **Ross A**, Hammerling U. Retinoids and the immune system. In: Sporn M, Roberts A, Goodman D, editors. The retinoids: Biology, chemistry and medicine. 2nd ed. New York: Raven Press, 1994: 597-630
  - 95 **Trinchieri G**. Biology of natural killer cells. *Adv Immunol* 1989; **47**: 187-376 [PMID: 2683611 DOI: 10.1016/S0065-2776(08)60664-1]
  - 96 **Michael A**, Hackett JJ, Bennett M, Kumar V, Yuan D. Regulation of B lymphocytes by natural killer cells. Role of IFN-gamma. *J Immunol* 1989; **142**: 1095-1101 [PMID: 2492576]
  - 97 **Alter BP**, Potter NU, Li FP. Classification and aetiology of the aplastic anaemias. *Clin Haematol* 1978; **7**: 431-465 [PMID: 363321]
  - 98 **Micksche M**, Colot M, Uchida A, Kokoschka EM, Lugar TA, Dittrich C, Moser K, Rainer H, Lanzhofer R, Kolb R, Jakesz R, Schemper M, Kakron O, Zwick H, Scheiner A, Flicker H, Sagaster P. Immunomodulation in cancer patients by synthetic biological response modifiers. *Cancer Treat Symp* 1985; **1**: 27-35
  - 99 **Santoni A**, Cerruti Sola S, Giovarelli M, Martinetto P, Vietti D, Forni G. Modulation of natural killer activity in mice by prolonged administration of various doses of dietary retinoids. *Nat Immun Cell Growth Regul* 1986; **5**: 259-266 [PMID: 3785241]
  - 100 **Fortes C**, Forastiere F, Agabiti N, Fano V, Pacifici R, Virgili F, Piras G, Guidi L, Bartoloni C, Tricerri A, Zuccaro P, Ebrahim S, Perucci CA. The effect of zinc and vitamin A supplementation on immune response in an older population. *J Am Geriatr Soc* 1998; **46**: 19-26 [PMID: 9434661 DOI: 10.1111/j.1532-5415.1998.tb01008.x]
  - 101 **Bowman TA**, Goonewardene IM, Pasatiempo AM, Ross AC, Taylor CE. Vitamin A deficiency decreases natural killer cell activity and interferon production in rats. *J Nutr* 1990; **120**: 1264-1273 [PMID: 1698953]
  - 102 **Bogomolski-Yahalom V**, Matzner Y. Disorders of neutrophil function. *Blood Rev* 1995; **9**: 183-190 [PMID: 8563520 DOI: 10.1016/0268-960X(95)90024-1]
  - 103 **Twining SS**, Schulte DP, Wilson PM, Fish BL, Moulder JE. Vitamin A deficiency alters rat neutrophil function. *J Nutr* 1997; **127**: 558-565 [PMID: 9109605]
  - 104 **Robertson KA**, Emami B, Mueller L, Collins SJ. Multiple members of the retinoic acid receptor family are capable of mediating the granulocytic differentiation of HL-60 cells. *Mol Cell Biol* 1992; **12**: 3743-3749 [PMID: 1324405 DOI: 10.1128/MCB.12.9.3743]
  - 105 **Tsai S**, Collins SJ. A dominant negative retinoic acid receptor blocks neutrophil differentiation at the promyelocyte stage. *Proc Natl Acad Sci USA* 1993; **90**: 7153-7157 [PMID: 8394011 DOI: 10.1073/pnas.90.15.7153]
  - 106 **Maun NA**, Gaines P, Khanna-Gupta A, Zibello T, Enriquez L, Goldberg L, Berliner N. G-CSF signaling can differentiate promyelocytes expressing a defective retinoic acid receptor: evidence for divergent pathways regulating neutrophil differentiation. *Blood* 2004; **103**: 1693-1701 [PMID: 14604978 DOI: 10.1182/blood-2002-10-3247]
  - 107 **Ribeiro OG**, Maria DA, Adriouch S, Pechberty S, Cabrera WH, Morisset J, Ibañez OM, Seman M. Convergent alteration of granulopoiesis, chemotactic activity, and neutrophil apoptosis during mouse selection for high acute inflammatory response. *J Leukoc Biol* 2003; **74**: 497-506 [PMID: 12960266 DOI: 10.1189/jlb.0103039]
  - 108 **Zhao Z**, Ross AC. Retinoic acid repletion restores the number of leukocytes and their subsets and stimulates natural cytotoxicity in vitamin A-deficient rats. *J Nutr* 1995; **125**: 2064-2073 [PMID: 7643240]
  - 109 **Higuchi H**, Nagahata H. Effects of vitamins A and E on superoxide production and intracellular signaling of neutrophils in Holstein calves. *Can J Vet Res* 2000; **64**: 69-75 [PMID: 10680660]
  - 110 **Bluestone JA**, Herold K, Eisenbarth G. Genetics, pathogenesis and clinical interventions in type 1 diabetes. *Nature* 2010; **464**: 1293-1300 [PMID: 20432533 DOI: 10.1038/nature08933]
  - 111 **Christianson SW**, Shultz LD, Leiter EH. Adoptive transfer of diabetes into immunodeficient NOD-scid/scid mice. Relative contributions of CD4+ and CD8+ T-cells from diabetic versus prediabetic NOD.NON-Thy-1a donors. *Diabetes* 1993; **42**: 44-55

- [PMID: 8093606 DOI: 10.2337/diab.42.1.44]
- 112 **Bendelac A**, Boitard C, Bedossa P, Bazin H, Bach JF, Carnaud C. Adoptive T cell transfer of autoimmune nonobese diabetic mouse diabetes does not require recruitment of host B lymphocytes. *J Immunol* 1988; **141**: 2625-2628 [PMID: 3262666]
  - 113 **Peterson JD**, Pike B, McDuffie M, Haskins K. Islet-specific T cell clones transfer diabetes to nonobese diabetic (NOD) F1 mice. *J Immunol* 1994; **153**: 2800-2806 [PMID: 8077683]
  - 114 **Lennon GP**, Bettini M, Burton AR, Vincent E, Arnold PY, Santamaria P, Vignali DA. T cell islet accumulation in type 1 diabetes is a tightly regulated, cell-autonomous event. *Immunity* 2009; **31**: 643-653 [PMID: 19818656 DOI: 10.1016/j.immuni.2009.07.008]
  - 115 **van Belle TL**, Coppieters KT, von Herrath MG. Type 1 diabetes: etiology, immunology, and therapeutic strategies. *Physiol Rev* 2011; **91**: 79-118 [PMID: 21248163 DOI: 10.1152/physrev.00003.2010]
  - 116 **Hamilton-Williams EE**, Palmer SE, Charlton B, Slattery RM. Beta cell MHC class I is a late requirement for diabetes. *Proc Natl Acad Sci USA* 2003; **100**: 6688-6693 [PMID: 12750472 DOI: 10.1073/pnas.1131954100]
  - 117 **Cardozo AK**, Ortis F, Stirling J, Feng YM, Rasschaert J, Tonnesen M, Van Eylen F, Mandrup-Poulsen T, Herchuelz A, Eizirik DL. Cytokines downregulate the sarcoendoplasmic reticulum pump Ca<sup>2+</sup> ATPase 2b and deplete endoplasmic reticulum Ca<sup>2+</sup>, leading to induction of endoplasmic reticulum stress in pancreatic beta-cells. *Diabetes* 2005; **54**: 452-461 [PMID: 15677503 DOI: 10.2337/diabetes.54.2.452]
  - 118 **Cnop M**, Welsh N, Jonas JC, Jörns A, Lenzen S, Eizirik DL. Mechanisms of pancreatic beta-cell death in type 1 and type 2 diabetes: many differences, few similarities. *Diabetes* 2005; **54** Suppl 2: S97-107 [PMID: 16306347 DOI: 10.2337/diabetes.54.suppl\_2.S97]
  - 119 **Conrad B**, Weidmann E, Trucco G, Rudert WA, Behboo R, Ricordi C, Rodriguez-Rilo H, Finegold D, Trucco M. Evidence for superantigen involvement in insulin-dependent diabetes mellitus aetiology. *Nature* 1994; **371**: 351-355 [PMID: 8090207 DOI: 10.1038/371351a0]
  - 120 **Bottazzo GF**, Dean BM, McNally JM, MacKay EH, Swift PG, Gamble DR. In situ characterization of autoimmune phenomena and expression of HLA molecules in the pancreas in diabetic insulinitis. *N Engl J Med* 1985; **313**: 353-360 [PMID: 3159965 DOI: 10.1056/NEJM198508083130604]
  - 121 **Iwata M**, Hirakiyama A, Eshima Y, Kagechika H, Kato C, Song SY. Retinoic acid imprints gut-homing specificity on T cells. *Immunity* 2004; **21**: 527-538 [PMID: 15485630 DOI: 10.1016/j.immuni.2004.08.011]
  - 122 **Bettelli E**, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, Weiner HL, Kuchroo VK. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* 2006; **441**: 235-238 [PMID: 16648838 DOI: 10.1038/nature04753]
  - 123 **Korn T**, Bettelli E, Gao W, Awasthi A, Jäger A, Strom TB, Oukka M, Kuchroo VK. IL-21 initiates an alternative pathway to induce proinflammatory T(H)17 cells. *Nature* 2007; **448**: 484-487 [PMID: 17581588 DOI: 10.1038/nature05970]
  - 124 **Korn T**, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 Cells. *Annu Rev Immunol* 2009; **27**: 485-517 [PMID: 19132915 DOI: 10.1146/annurev.immunol.021908.132710]
  - 125 **Mucida D**, Park Y, Kim G, Turovskaya O, Scott I, Kronenberg M, Cheroutre H. Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. *Science* 2007; **317**: 256-260 [PMID: 17569825 DOI: 10.1126/science.1145697]
  - 126 **Mangan PR**, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, Hatton RD, Wahl SM, Schoeb TR, Weaver CT. Transforming growth factor-beta induces development of the T(H)17 lineage. *Nature* 2006; **441**: 231-234 [PMID: 16648837 DOI: 10.1038/nature04754]
  - 127 **Fragoso YD**, Stoney PN, McCaffery PJ. The evidence for a beneficial role of vitamin A in multiple sclerosis. *CNS Drugs* 2014; **28**: 291-299 [PMID: 24557746 DOI: 10.1007/s40263-014-0148-4]
  - 128 **Leal JY**, Castejón HV, Romero T, Ortega P, Gómez G, Amaya D, Estévez J. [Serum values of cytokines in children with vitamin A deficiency disorders]. *Invest Clin* 2004; **45**: 243-256 [PMID: 15469069]
  - 129 **Stephensen CB**. Vitamin A, infection, and immune function. *Annu Rev Nutr* 2001; **21**: 167-192 [PMID: 11375434 DOI: 10.1146/annurev.nutr.21.1.167]
  - 130 **Asano M**, Toda M, Sakaguchi N, Sakaguchi S. Autoimmune disease as a consequence of developmental abnormality of a T cell subpopulation. *J Exp Med* 1996; **184**: 387-396 [PMID: 8760792 DOI: 10.1084/jem.184.2.387]
  - 131 **Amrani A**, Verdaguer J, Thiessen S, Bou S, Santamaria P. IL-1alpha, IL-1beta, and IFN-gamma mark beta cells for Fas-dependent destruction by diabetogenic CD4(+) T lymphocytes. *J Clin Invest* 2000; **105**: 459-468 [PMID: 10683375 DOI: 10.1172/JCI18185]
  - 132 **Savinov AY**, Wong FS, Chervonsky AV. IFN-gamma affects homing of diabetogenic T cells. *J Immunol* 2001; **167**: 6637-6643 [PMID: 11714835 DOI: 10.4049/jimmunol.167.11.6637]
  - 133 **Wang B**, André I, Gonzalez A, Katz JD, Aguet M, Benoist C, Mathis D. Interferon-gamma impacts at multiple points during the progression of autoimmune diabetes. *Proc Natl Acad Sci USA* 1997; **94**: 13844-13849 [PMID: 9391115 DOI: 10.1073/pnas.94.25.13844]
  - 134 **Suarez-Pinzon W**, Rajotte RV, Mosmann TR, Rabinovitch A. Both CD4+ and CD8+ T-cells in syngeneic islet grafts in NOD mice produce interferon-gamma during beta-cell destruction. *Diabetes* 1996; **45**: 1350-1357 [PMID: 8826970 DOI: 10.2337/diab.45.10.1350]
  - 135 **Haskins K**, McDuffie M. Acceleration of diabetes in young NOD mice with a CD4+ islet-specific T cell clone. *Science* 1990; **249**: 1433-1436 [PMID: 2205920 DOI: 10.1126/science.2205920]
  - 136 **Wicker LS**, Leiter EH, Todd JA, Renjilian RJ, Peterson E, Fischer PA, Podolin PL, Zijlstra M, Peterson LB.  $\beta$ 2-microglobulin-deficient NOD mice do not develop insulinitis or diabetes. *Diabetes* 1994; **43**: 500-504 [DOI: 10.2337/diab.43.3.500]
  - 137 **Santamaria P**. Effector lymphocytes in islet cell autoimmunity. *Rev Endocr Metab Disord* 2003; **4**: 271-280 [PMID: 14501178 DOI: 10.1023/A:1025156413404]
  - 138 **Zunino SJ**, Storms DH, Stephensen CB. Diets rich in polyphenols and vitamin A inhibit the development of type I autoimmune diabetes in nonobese diabetic mice. *J Nutr* 2007; **137**: 1216-1221 [PMID: 17449584]
  - 139 **Weaver CT**, Hatton RD, Mangan PR, Harrington LE. IL-17 family cytokines and the expanding diversity of effector T cell lineages. *Annu Rev Immunol* 2007; **25**: 821-852 [PMID: 17201677 DOI: 10.1146/annurev.immunol.25.022106.141557]
  - 140 **Van YH**, Lee WH, Ortiz S, Lee MH, Qin HJ, Liu CP. All-trans retinoic acid inhibits type 1 diabetes by T regulatory (Treg)-dependent suppression of interferon-gamma-producing T-cells without affecting Th17 cells. *Diabetes* 2009; **58**: 146-155 [PMID: 18984738 DOI: 10.2337/db08-1154]
  - 141 **Schambach F**, Schupp M, Lazar MA, Reiner SL. Activation of retinoic acid receptor-alpha favours regulatory T cell induction at the expense of IL-17-secreting T helper cell differentiation. *Eur J Immunol* 2007; **37**: 2396-2399 [PMID: 17694576 DOI: 10.1002/eji.200737621]
  - 142 **Kinoshita K**, Yoo BS, Nozaki Y, Sugiyama M, Ikoma S, Ohno M, Funauchi M, Kanamaru A. Retinoic acid reduces autoimmune renal injury and increases survival in NZB/W F1 mice. *J Immunol* 2003; **170**: 5793-5798 [PMID: 12759464 DOI: 10.4049/jimmunol.170.11.5793]
  - 143 **Xu J**, Storer PD, Chavis JA, Racke MK, Drew PD. Agonists for the peroxisome proliferator-activated receptor-alpha and the retinoid X receptor inhibit inflammatory responses of microglia. *J Neurosci Res* 2005; **81**: 403-411 [PMID: 15968640 DOI: 10.1002/jnr.20518]
  - 144 **Shidfar F**, Aghasi M, Vafa M, Heydari I, Hosseini S, Shidfar S. Effects of combination of zinc and vitamin A supplementation on serum fasting blood sugar, insulin, apolipoprotein B and apolipoprotein A-I in patients with type I diabetes. *Int J Food Sci Nutr* 2010; **61**: 182-191 [PMID: 20151940 DOI: 10.3109/09637480903334171]

- 145 **Zorena K**, Myśliwska J, Myśliwiec M, Balcerska A, Lipowski P, Raczynska K. Interleukin-12 and tumour necrosis factor- $\alpha$  equilibrium is a prerequisite for clinical course free from late complications in children with type 1 diabetes mellitus. *Scand J Immunol* 2008; **67**: 204-208 [PMID: 18201373 DOI: 10.1111/j.1365-3083.2007.02054.x]
- 146 **Arnold G**. Mouse Study Shows Hope for Vitamin A and Type 1 Diabetes. Available from: <http://pitchingdoc.com/fileupload/NOW%20Foods%20Articles/Diabetes/Mouse%20Study%20Show%20Hope%20for%20Vitamin%20A%20In%20Type%201%20Diabetes.pdf>
- 147 **Arimilli S**, Ferlin W, Solvason N, Deshpande S, Howard M, Mocci S. Chemokines in autoimmune diseases. *Immunol Rev* 2000; **177**: 43-51 [PMID: 11138783 DOI: 10.1034/j.1600-065X.2000.17716.x]
- 148 **Martin S**, van den Engel NK, Vinke A, Heidenthal E, Schulte B, Kolb H. Dominant role of intercellular adhesion molecule-1 in the pathogenesis of autoimmune diabetes in non-obese diabetic mice. *J Autoimmun* 2001; **17**: 109-117 [PMID: 11591119 DOI: 10.1006/jaut.2001.0526]
- 149 **Elias KM**, Laurence A, Davidson TS, Stephens G, Kanno Y, Shevach EM, O'Shea JJ. Retinoic acid inhibits Th17 polarization and enhances FoxP3 expression through a Stat-3/Stat-5 independent signaling pathway. *Blood* 2008; **111**: 1013-1020 [PMID: 17951529 DOI: 10.1182/blood-2007-06-096438]
- 150 **Sun CM**, Hall JA, Blank RB, Bouladoux N, Oukka M, Mora JR, Belkaid Y. Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid. *J Exp Med* 2007; **204**: 1775-1785 [PMID: 17620362 DOI: 10.1084/jem.20070602]
- 151 **Fujino S**, Andoh A, Bamba S, Ogawa A, Hata K, Araki Y, Bamba T, Fujiyama Y. Increased expression of interleukin 17 in inflammatory bowel disease. *Gut* 2003; **52**: 65-70 [PMID: 12477762 DOI: 10.1136/gut.52.1.65]
- 152 **Batten M**, Li J, Yi S, Kljavin NM, Danilenko DM, Lucas S, Lee J, de Sauvage FJ, Ghilardi N. Interleukin 27 limits autoimmune encephalomyelitis by suppressing the development of interleukin 17-producing T cells. *Nat Immunol* 2006; **7**: 929-936 [PMID: 16906167 DOI: 10.1038/ni1375]
- 153 **Koenders MI**, Lubberts E, Oppers-Walgreen B, van den Bersselaar L, Helsen MM, Di Padova FE, Boots AM, Gram H, Joosten LA, van den Berg WB. Blocking of interleukin-17 during reactivation of experimental arthritis prevents joint inflammation and bone erosion by decreasing RANKL and interleukin-1. *Am J Pathol* 2005; **167**: 141-149 [PMID: 15972960 DOI: 10.1016/S0002-9440(10)62961-6]
- 154 **Miyagawa N**, Homma T, Kagechika H, Shudo K, Nagai H. Effect of synthetic retinoid, TAC-101, on experimental autoimmune disease. *Pharmacology* 2003; **67**: 21-31 [PMID: 12444300 DOI: 10.1159/000066783]
- 155 **Osanai M**, Nishikiori N, Murata M, Chiba H, Kojima T, Sawada N. Cellular retinoic acid bioavailability determines epithelial integrity: Role of retinoic acid receptor  $\alpha$  agonists in colitis. *Mol Pharmacol* 2007; **71**: 250-258 [PMID: 17035595 DOI: 10.1124/mol.106.029579]
- 156 **Driscoll HK**, Chertow BS, Jelic TM, Baltaro RJ, Chandor SB, Walker EM, Dadgari JM, Pofahl AB. Vitamin A status affects the development of diabetes and insulinitis in BB rats. *Metabolism* 1996; **45**: 248-253 [PMID: 8596498 DOI: 10.1016/S0026-0495(96)90062-1]

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## Neuroendocrine hormone amylin in diabetes

Xiao-Xi Zhang, Yan-Hong Pan, Yan-Mei Huang, Hai-Lu Zhao

Xiao-Xi Zhang, Yan-Hong Pan, Yan-Mei Huang, Hai-Lu Zhao, Centre of Diabetic Systems Medicine, Guangxi Key Laboratory of Excellence, Guilin Medical University, Guilin 541004, Guangxi Zhuang Autonomous Region, China

Xiao-Xi Zhang, Hai-Lu Zhao, Institute of Basic Medical Science, College of Basic Medical Science, Guilin Medical University, Guilin 541004, Guangxi Zhuang Autonomous Region, China

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**Correspondence to:** Hai-Lu Zhao, MD, PhD, Professor, Director, Centre of Diabetic Systems Medicine, Guangxi Key Laboratory of Excellence, Guilin Medical University, Huan Cheng North 2<sup>nd</sup> Road 109, Guilin 541004, Guangxi Zhuang Autonomous Region, China. [zhaohailu9@126.com](mailto:zhaohailu9@126.com)  
Telephone: +86-773-5805803  
Fax: +86-773-5895805

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### Abstract

The neuroendocrine hormone amylin, also known as islet amyloid polypeptide, is co-localized, co-packaged and co-secreted with insulin from adult pancreatic islet  $\beta$  cells to maintain glucose homeostasis. Specifically, amylin reduces secretion of nutrient-stimulated glucagon, regulates blood pressure with an effect on renin-angiotensin system, and delays gastric emptying. The physiological actions of human amylin attribute to the conformational  $\alpha$ -helix monomers whereas the misfolding instable oligomers may be detrimental to the islet  $\beta$  cells and further transform to  $\beta$ -sheet fibrils as amyloid deposits. No direct evidence proves that the amylin fibrils in amyloid deposits cause diabetes. Here we also have performed a systematic review of human amylin gene changes and reported the S20G mutation is minor in the development of diabetes. In addition to the metabolic effects, human amylin may modulate autoimmunity and innate inflammation through regulatory T cells to impact on both human type 1 and type 2 diabetes.

**Key words:** Amylin; Neuroendocrine hormone; Diabetes

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**Core tip:** This is a systematic review to describe amylin as a neuroendocrine hormone. Besides the glucose homeostasis and cytotoxicity of amylin, we tried to perform that the S20G mutation of human amylin is also minor in the pathogenesis of diabetes. In addition to the metabolic effects, human amylin may have impact on autoimmunity, implicating a potential as the immunosuppressor to improve autoimmunity conditions in the future therapy of diabetes, allergic diseases and immune rejection.

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## INTRODUCTION

Amylin, or islet amyloid polypeptide (IAPP), is a neuroendocrine hormone co-localized, co-secreted and co-packaged with insulin from pancreatic  $\beta$  cells<sup>[1,2]</sup>. Abnormalities in human amylin folding, secretion and action have detrimental effects on islet function and glucose regulation by islet amyloidosis and  $\beta$  cell dysfunction in type 2 diabetes (T2D)<sup>[3-5]</sup>. The molecules of amylin polypeptide fold to form the  $\alpha$ -helix monomers and oligomers and the  $\beta$ -sheet fibrils. The amylin-aggregated amyloid fibrils are thought to form through smaller cell toxic intermediates and deposited amyloid disrupts normal islet architecture<sup>[6]</sup>. However, amylin plays a critical role in metabolism homeostasis<sup>[7]</sup> as a neuroendocrine hormone that carries a targeted signal to the brain. Several actions of amylin that impact glucose regulation have been identified, including the effects on nutrient-stimulated glucagon secretion<sup>[8]</sup>, on nutrient delivery from the stomach to the small intestine for absorption<sup>[9]</sup>, on renin-angiotensin system (RAS)<sup>[10]</sup> and on food intake by delaying gastric emptying<sup>[11]</sup>.

## FUNCTIONAL AMYLIN

Amylin functions as part of the neuroendocrine pancreas and contributes to glucose homeostasis with other two pancreatic islet hormones insulin and glucagon. The amylin and insulin is a pair of synergistic partner genes co-expressed by a common promoter<sup>[12]</sup>, and regulates the levels of glucose by complex endocrine and neuronal pathways. In physiological state, the simultaneous release of amylin and insulin from the secretory granules results in a parallel pattern in the islet  $\beta$ -cells in response to glucose stimulation<sup>[13]</sup>. However, concentration of plasma amylin and insulin decreased in advanced T2D<sup>[7]</sup>. Glucagon commonly increases blood glucose when nutrients are not available; while insulin and amylin primarily decrease the post-meal glucose by stimulating the uptake of glucose from circulation into muscle and fat cells for storage and by inhibiting the endogenous glucose output from liver. Complimentary to insulin, amylin regulates postprandial glycaemia by suppressing postmeal glucagon secretion from islet  $\alpha$ -cells<sup>[8]</sup>, which is possibly mediated by signals from the vagus nerve at the pancreatic islets. Amylin and insulin also coordinate storage of carbohydrate to transfer triglyceride into muscle glycogen in skeletal muscles<sup>[14]</sup> probably by phosphorylase activation<sup>[15]</sup> (Table 1 and Figure 1).

As a neuroendocrine hormone, amylin also acts in the central nervous system to produce satiety through brainstem-localized receptors, which have been found at several locations in the brain, including the nucleus accumbens, the dorsal raphe and the area postrema in rat brain<sup>[16]</sup>. The area postrema may be an important

**Table 1 Physiological actions of amylin**

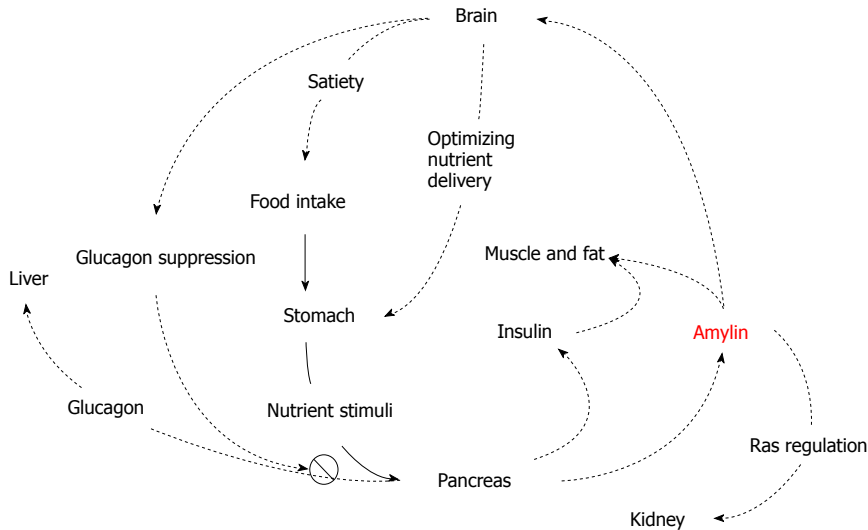
Neuroendocrine effects
Inhibiting insulin secretion in a high concentration
Inhibiting glucagon secretion at mealtime
Delaying nutrient delivery from stomach to the small intestine
Reducing food intake by a signal from the central nervous system
Metabolic effects
Co-regulating glucose with insulin and glucagon
Inhibiting muscle glycogen synthesis <sup>[15]</sup>
Stimulating oxidative responses and low density lipoprotein uptake in insulin-producing cells
Inhibiting bone resorption
Lipolytic-like effects
Renal effects
Renin $\uparrow$
Angiotensin II $\uparrow$
Regulating renal growth
Regulating water-sodium homeostasis
Haemodynamic effect
Aldosterone $\uparrow$
Hypocalcaemia
Vasodilation

site for amylin action. This area does not have a blood brain barrier and allows access to circulatory peptides. Lesioning studies have indicated that some of amylin's actions are mediated at this site. The suppression of neuronal amylin on food intake and gastrointestinal motility<sup>[17]</sup> to slow down the absorption and to limit the rate at which glucose enters the circulation<sup>[18]</sup> has been found in human. Gastric emptying is considered to be a typical pathological phenomenon and a crucial reason for the postprandial hyperglycemia in T1D. It is believed that most of amylin deficiency in T1D may be pathogenically significant in the gastric behavior<sup>[19]</sup>. Thus, high plasma amylin concentration in young with newly-diagnosed T1D<sup>[20]</sup>, which may result in a delay in gastric emptying that markedly improved postprandial glucose excursions in new T1D patients<sup>[19]</sup>. Amylin deficiency significantly affects the lack of delay in gastric emptying in response to hyperglycemia in T1D<sup>[19]</sup>, and is further supported by the highly potent protective effects of amylin on glucose homeostasis<sup>[21]</sup>.

Furthermore, a physiological effect of amylin on the RAS has been implicated in the hemodynamic regulation of blood pressure<sup>[22]</sup> and kidney function<sup>[10]</sup>. Inhibition of angiotensin-converting enzyme (ACE) is associated with the reducing density of amylin binding in the renal cortex<sup>[10]</sup>. Pharmacokinetics pattern of amylin closely resembles that of C-peptide<sup>[23-25]</sup>. Excreted in the urine, when glomerular filtration decreases, amylin cumulates in the blood stream. Therefore, patients with renal failure may have high levels of circulating amylin<sup>[26]</sup>. These patients also have a higher than normal prevalence of islet amyloid in the absence of diabetic symptoms<sup>[27]</sup> (Table 1 and Figure 1).

## KINETICS OF AMYLIN

Human amylin is derived from a larger precursor



**Figure 1 Overview of physiological actions of amylin.** (1) Amylin suppresses glucagon secretion from islet alpha cells at mealtime and thus, inhibits glucagons-induced glucose release from the liver; (2) Amylin delays nutrient delivery from the stomach to the small intestine for absorption; (3) Amylin reduces food intake by a signal mediated through the central nervous system; (4) Renal amylin may stimulate Renin-Angiotensin System; and (5) Amylin and insulin coordinate storage of carbohydrate.

proamylin, coding sequence with 89 amino acids residue. The flanking peptides at N-terminal and C-terminal of mature amylin are removed by a proteolytic enzyme, which is also responsible for proinsulin to insulin conversion in the  $\beta$  cells<sup>[28]</sup>. The prohormone convertases PC2 and PC3 involved in processing proinsulin are likely responsible for amylin processing as well<sup>[5]</sup>.

Measuring the concentration of circulating amylin is challenging. The minimum detectable concentration of amylin in 50 mL plasma is 0.5 to 2 pmol/L, and the dynamic range is 2 to 100 pmol/L<sup>[29]</sup>. The basal plasma concentrations of amylin in human in the 2-15 pmol/L range, with an insulin/amylin ratio of 10-100:1<sup>[30,31]</sup>. In healthy subjects, circulating amylin rises in response to the glucose challenge<sup>[32]</sup>. The amylin to insulin molar ratio is similar at all time points despite of high-frequency oscillations and inter-race differences in circulating amylin concentrations<sup>[33,34]</sup>.

Circulating amylin levels are increased in individuals with obesity, hypertension, positive family history of insulin resistance in line with hyperinsulinaemia<sup>[35-37]</sup>. An exaggerated amylin response has been documented in subjects with obesity and impaired glucose tolerance<sup>[32,36]</sup>. Moreover, the amylin to insulin ratio is consistent in insulin-resistant persons at all time points, although large interindividual variations (0.2% to 1.6%) in amylin/insulin secretory ratios have been documented<sup>[35]</sup>. Thus, hyperamylinemia due to insulin resistance precedes the occurrence of T2D<sup>[36]</sup>. However, in later-stage T2D, the secretion of both amylin and insulin becomes deficient<sup>[32]</sup>. In diabetic patients on insulin treatment, amylin/insulin is detectable but the response to the glucose challenge is negligible, reflecting functional failure of the islet  $\beta$  cells<sup>[38]</sup>. Interestingly, diabetes is also characterised by an excess of glucagon, in particularly after mealtimes. The net effect of insulin and amylin deficiency and glucagon excess is an increased postmeal glucose level. Furthermore, prolonged exposure of pancreatic islets to hyperglycaemia favours selective amylin secretion, increasing the risk of islet amyloid formation and  $\beta$  cell

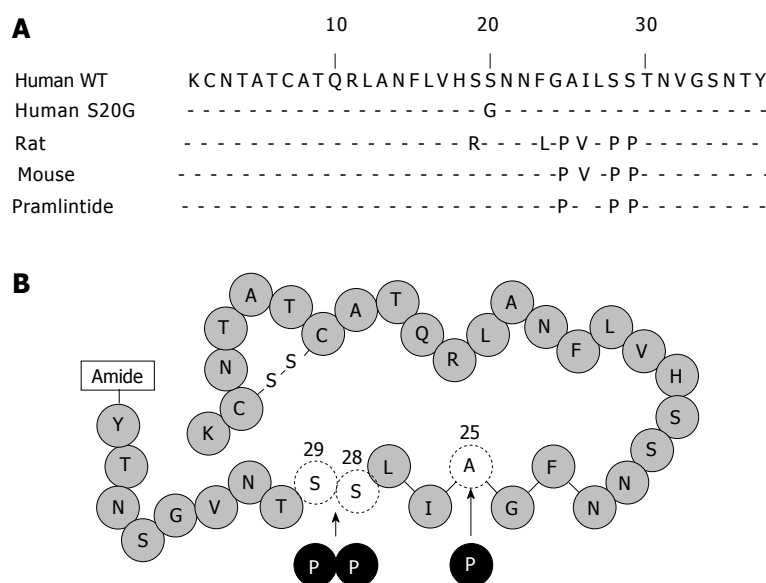
apoptosis<sup>[39,40]</sup>.

## AMYLIN-DERIVED AMYLOIDOSIS

Amyloidosis is a generic term for aggregation state of amyloid polypeptide with  $\beta$ -sheet conformation that bounds to each other by certain chemical bonds<sup>[41]</sup>. Amylin is encoded by calcitonin mRNA from a gene made up of three introns on the 12<sup>th</sup> chromosome<sup>[42]</sup>. Besides amylin, more than 25 proteins in human are known by their fibrillate aggregation<sup>[43,44]</sup>. Many of them have similar protein structures with amyloid-like properties and characteristic occurrences in metabolic disturbances, such as amylin, amyloid light-chain and  $\beta$ -amyloid<sup>[45,46]</sup>. In the islet of T2D patients, amylin fibrils commonly contribute to the form of islet amyloid. In addition, amylin is also found to deposit in brain<sup>[16]</sup>, plays the potential role in the development of Alzheimer's disease (AD) and cerebrovascular disease (CVD) pathology with  $\beta$ -amyloid, or might impair brain function independently of  $\beta$ -amyloid pathology<sup>[47]</sup>.

Certain gene mutations, amino acid sites in amylin protein and minor components are more or less associated with amyloid deposits. It has been reported some mutations in the human amylin gene leading to amino acid substitutions, such as S20G. S20G is an important amylin gene mutation resulting in a glycine for serine substitution at position 20 of the mature IAPP molecule (Figure 2A). *In vitro* studies indicate that the S20G mutation amylin is more cytotoxic in forming amyloid and inducing apoptosis in COS-1 cells<sup>[48]</sup>. A low prevalence (< 5%) of the S20G has been reported in T2D Japanese<sup>[49-51]</sup>, Hong Kong Chinese<sup>[52,53]</sup>, Taiwanese<sup>[54]</sup>, and Mainland Chinese. All the cases with the mutation S20G are heterozygous. Although the heterozygous mutation S20G is more common in diabetic patients than in normal control (2.6% vs 0.9%,  $P < 0.0001$ ), linkage analysis reveals that mutation in or near amylin gene is unlikely a major course of T2D<sup>[55]</sup> (Table 2).

Many molecular chaperones, like apolipoprotein E



**Figure 2 Amino acid sequence and diagrammatic representations of human amylin and pramlintide.** A: Amino acid sequence alignment of human (WT, S20G), rat, mouse amylin and pramlintide. Only the amino acids that differ are shown. The sequence between amino acids 20 to 29 represents the amyloidogenic domain; B: The synthetic amylin analog pramlintide differs from human amylin at three amino acid sites (proline at 25, 28, and 29) and this molecule overcomes these disadvantages of human amylin.

(apoE) and heat shock protein (HSP) family, may relate to amylin deposits<sup>[56-58]</sup>, and is generally considered as a major genetic modulator of  $\beta$ -amyloid deposition and risk of AD<sup>[59,60]</sup>. The apoE  $\epsilon$ 4 allele particularly affects the increased risk for atherosclerosis<sup>[61]</sup>, brain plaque<sup>[61]</sup> and islet amyloidosis. In T2D, apoE plays a critical role in lipid metabolism, amylin fibril formation<sup>[62]</sup> and is a probable link to atherosclerosis<sup>[57]</sup>. HSP is identified within highly purified  $\beta$  cell granules derived from INS-1E islet  $\beta$  cells such as insulin and amylin<sup>[63]</sup>. Vita demonstrates the existence of direct functional interactions involved HSP70, can suppress the misfolding of human amylin<sup>[58]</sup>, which is also proved to limit the toxicity of  $\beta$ -amyloid<sup>[64]</sup>. These chaperones may contribute the AD-diabetes link at the pathophysiological level, including the interactive amyloid of  $\beta$ -amyloid and human amylin<sup>[65,66]</sup>.

## CONFORMATIONS OF AMYLIN

The conformation of amylin is considered a significant factor in that abnormal accumulation of amylin fibrils in organs may lead to amyloidosis in T2D. Human amylin is subtyped into three different conformations: Monomers, oligomers and fibrils (Figure 3). Monomers are unfolded random-coiled peptides physiologically. These molecules can be misfolding with  $\alpha$ -helix structures, and aggregated into the pathologic oligomers, the soluble amyloid intermediates, which include spherical particles of 2.7 to 4.2 nm in diameter<sup>[67]</sup>. Amylin fibrils formation is a self-driven process accumulating the misfolded oligomeric proteins with a  $\beta$ -sheet fibrillar structure into insoluble islet amyloid deposits. Islet amylin-amyloid is the pathologic hallmark of most individuals with T2D<sup>[22,68]</sup>.

In physiological state, the toxic oligomers can be rescued into amyloid fibrils by chaperones or eliminated by the ubiquitin-proteasome system<sup>[69]</sup>. Concentration of amylin fibrils in the intracytoplasmic organelles of human beta cells far exceed the *in vitro* concentration

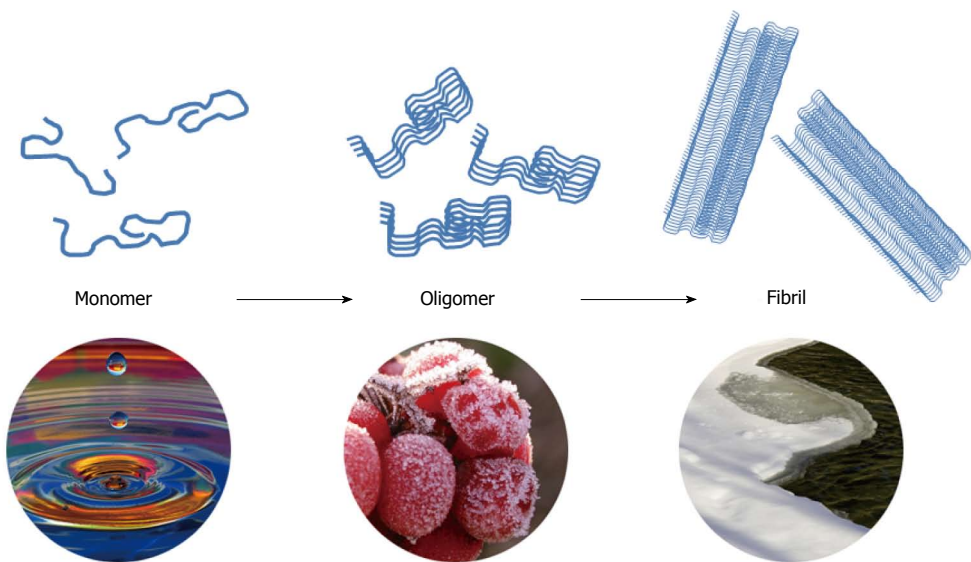
required for amyloid formation, so there must be an underlying mechanism in normal beta cells to induce the aggregation of amylin monomers into fibrils. Mechanisms that may associate with amylin-amyloid include an acid pH<sup>[70]</sup>, the presence of chaperon proteins (HSP), or the presence of other proteins<sup>[58]</sup>. Then these possible factors for amylin aggregation may derive from hyperglycaemia, high-fat diet, or low-grade chronic inflammation<sup>[3]</sup>, which are considered as the cardinal symptoms of T2D. The amylin monomer has its special function in endocrine system, or further polymerise to amyloid fibrils, which may play an important role in cell informational transfer, memory and survival prolonging<sup>[71]</sup>.

Mature fibrillar aggregate of amylin has been considered to be nontoxic, and even these small amyloid deposits seen widely located in islets or other organs in T2D, may not have significant contribution to organs damage. Therefore, the formation of fibrils from cytotoxic oligomers can be considered as a protective mechanism of transforming a dynamic protein into inert amyloid. Here we have to be curious whether this process of fibrillar formation initially acts as a rescuer in the pathway of cell failure<sup>[71]</sup>.

The amino acid sequence of amylin derived from islet amyloid in T2D is identical to that present in healthy humans, and amylin from human insulinoma tissue. Moreover, amylin structure exhibits close sequence homology among all species in both the amino terminal (residues 1 to 19) and the carboxy terminal (residues 20 to 29) regions. In contrast, residues 20 to 29 show considerable divergence among species and have been implicated in the conversion of the peptide's secondary structure from a predominantly  $\alpha$  helical one to a  $\beta$  sheet structure<sup>[72]</sup>. This assignment is based on the comparison of the sequences of human amylin, which is highly amyloidogenic, with those of cat amylin, which is moderately amyloidogenic, and with rat and mouse amylin, which does not aggregate to form amyloid<sup>[72,73]</sup>.

**Table 2** Prevalence of the amylin gene mutation S20G in Asian populations (%) *n*

	Ref.	Type 2 diabetes	Impaired glucose tolerance	Type 1 diabetes	Control
Japanese	Seino <i>et al</i> <sup>[50]</sup>	2.6 (1538)	-	-	0.8 (1108)
Japanese	Yamada <i>et al</i> <sup>[51]</sup>	4.7 (86)	-	-	1.6 (182)
Japanese	Sakagashira <i>et al</i> <sup>[49]</sup>	4.1 (294)	-	0 (59)	0 (187)
Hong Kong Chinese	Ng <i>et al</i> <sup>[53]</sup>	1.5 (462)	-	-	0 (126)
Taiwanese	Chuang <i>et al</i> <sup>[54]</sup>	1.6 (182)	4.2 (24)	1.6 (122)	4.4 (99)
Mainland Chinese	Lee <i>et al</i> <sup>[52]</sup>	2.1 (94)	-	-	0 (106)



**Figure 3** Three conformations of human amylin. Monomers of amylin with physiological functions mainly contribute to glucose and lipid homeostasis and tend to misfold into the cytotoxic oligomers. A self-driven process is accumulating the misfolded oligomers into insoluble nontoxic amylin fibrils with a  $\beta$ -sheet structure.

### AMYLIN TOXICITY

Mechanisms of islet  $\beta$  cell depletion by amyloid include mechanical replacement, apoptosis, necrosis and  $\beta$  cell membrane damage. Human amylin has been clearly shown toxic to insulin-producing  $\beta$  cells of the adult pancreas of rats and humans<sup>[74]</sup>. The cytotoxic action of amylin in insulin-producing cells is paralleled by increased oxidative responses and low density lipoprotein (LDL) uptake, suggesting that cytotoxic mechanisms of amylin in insulin-producing cells involve changes in pathways of cellular oxidative stress systems and lipid homeostasis<sup>[75]</sup>.

Soluble oligomer of amylin is recently reported to contribute the primary toxicity in T2D but not insoluble fibril in the amyloid diseases<sup>[76-78]</sup>. Different misfolded oligomers of amylin with a conformation-dependent structure suggest that they share a common mechanism of pathogenesis<sup>[79]</sup>. Like oligomer of  $\beta$  amyloid protein playing an important role in the pathogenesis of AD and CVD<sup>[80]</sup>, oligomeric amylin is also a central subject in the risk of the islet  $\beta$ -cell lesion in T2D through formation of toroidal (ion-leaking) pores inserted into membranes<sup>[81,82]</sup>.

### THERAPEUTIC APPLICATION AND PROSPECTIVE

Human amylin has a tendency to aggregate, form

insoluble particles and stick to surfaces. Learnt from non-amyloidogenic rat amylin, the peptide structure is broken by substituting the positions 25 alanine, 28 and 29 serines into proline residues (Figure 2B). This analog of human amylin, named "pramlintide", is used for the potential prevention of complications of T1D as an adjunct with insulin and a single agent for T2D<sup>[2]</sup>. This soluble, stable synthetic analog amylin avoids aggregation of amyloid relating the development of  $\beta$ -cell dysfunction<sup>[83]</sup>. Like wild-type human amylin, pramlintide can adjust postprandial glucagon release and gastric emptying rate in individuals with T1D and T2D<sup>[84-87]</sup>. In clinical therapy of diabetes, pramlintide as an assistant treatment of insulin usually decreases postprandial glucose without rising insulin level<sup>[88,89]</sup>. In T1D, pramlintide therapy significantly reduced 4.4-6.6 mmol/mol haemoglobin a1c at 26 wk vs placebo<sup>[90,91]</sup>. And mean body weight was significantly reduced (-0.8 to -1.3 kg at week 26 or 29) vs placebo<sup>[90-92]</sup>. Then in T2D, pramlintide therapy resulted in significant reductions in haemoglobin a1c (-7.7 to -8.7 mmol/mol after 16 or 26 wk) and mean body weight (-1.4 to -1.6 kg after 16 or 26 wk) vs placebo<sup>[93-95]</sup>. According to these functions, the pramlintide is manufactured and designed as injection in pen injector. Since the pH value of pramlintide buffer is incompatible with most insulin products, pramlintide is recommended not to mix with insulin in the same syringe (shown in Symlin<sup>®</sup> Package Insert). This analog



has the same functions of blood glucose regulation and gastric emptying delay as wild-type human amylin.

Human amylin no doubt plays a significant role in neuroendocrine contribution to glucose homeostasis. Treatment with non-fibrillar pramlintide improves glycaemic control and weight management without adverse events of severe hypoglycaemia in T1D and T2D<sup>[96]</sup>. However, whether the toxicity of fibrillar amylin contributes significantly to pathogenesis of diabetes is yet unconvincing. Studies data indicate that microscopically evident fibrillar amylin is neither necessary nor sufficient to cause diabetes, but rather that it is positively correlated with protection<sup>[97,98]</sup>.

It is worth noting that amylin may regulate the inflammatory response and immune factor secretion<sup>[99,100]</sup>. Mouse amylin was reported that can trigger a broad autoimmune response by CD4<sup>+</sup> effector T cells in NOD mice<sup>[101]</sup>. Recent study shows that human amylin can induce CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> Regulatory T cells and reduce risk of autoimmune diabetes<sup>[102]</sup>. It firstly demonstrates autoimmune inhibition by human amylin. All these findings suggest a novel approach to restore glucose homeostasis and improve autoimmunity conditions such as autoimmune diseases, allergic diseases, immune rejection of organ transplantation and graft vs host reaction (GVHR).

## REFERENCES

- 1 **Westermarck P**, Wernstedt C, Wilander E, Sletten K. A novel peptide in the calcitonin gene related peptide family as an amyloid fibril protein in the endocrine pancreas. *Biochem Bioph Res Co* 1986; **140**: 827-831 [DOI: 10.1016/0006-291X (86)90708-4]
- 2 **Cooper GJ**, Willis AC, Clark A, Turner RC, Sim RB, Reid KB. Purification and characterization of a peptide from amyloid-rich pancreases of type 2 diabetic patients. *Proc Natl Acad Sci USA* 1987; **84**: 8628-8632 [PMID: 3317417 DOI: 10.1073/pnas.84.23.8628]
- 3 **Clark A**, Chargé SB, Badman MK, de Koning EJ. Islet amyloid in type 2 (non-insulin-dependent) diabetes. *APMIS* 1996; **104**: 12-18 [PMID: 8645452 DOI: DOI: 10.1111/j.1699-0463.1996.tb00680.x]
- 4 **Clark A**, Charge SB, Badman MK, MacArthur DA, de Koning EJ. Islet amyloid polypeptide: actions and role in the pathogenesis of diabetes. *Biochem Soc Trans* 1996; **24**: 594-599 [PMID: 8736810 DOI: 10.1042/bst0240594]
- 5 **Kahn SE**, Andrikopoulos S, Verchere CB. Islet amyloid: a long-recognized but underappreciated pathological feature of type 2 diabetes. *Diabetes* 1999; **48**: 241-253 [PMID: 10334297 DOI: 10.2337/diabetes.48.2.241]
- 6 **Anguiano M**, Nowak RJ, Lansbury PT. Protofibrillar islet amyloid polypeptide permeabilizes synthetic vesicles by a pore-like mechanism that may be relevant to type II diabetes. *Biochemistry* 2002; **41**: 11338-11343 [PMID: 12234175 DOI: 10.1021/bi020314u]
- 7 **Kahn SE**, Verchere CB, Andrikopoulos S, Asberry PJ, Leonetti DL, Wahl PW, Boyko EJ, Schwartz RS, Newell-Morris L, Fujimoto WY. Reduced amylin release is a characteristic of impaired glucose tolerance and type 2 diabetes in Japanese Americans. *Diabetes* 1998; **47**: 640-645 [PMID: 9568698 DOI: 10.2337/diabetes.47.4.640]
- 8 **Gedulin BR**, Rink TJ, Young AA. Dose-response for glucagonostatic effect of amylin in rats. *Metabolism* 1997; **46**: 67-70 [PMID: 9005972 DOI: 10.1016/S0026-0495(97)90170-0]
- 9 **Edelman SV**, Weyer C. Unresolved challenges with insulin therapy in type 1 and type 2 diabetes: potential benefit of replacing amylin, a second beta-cell hormone. *Diabetes Technol Ther* 2002; **4**: 175-189 [PMID: 12079621 DOI: 10.1089/15209150260007390]
- 10 **Wookey PJ**, Cao Z, Cooper ME. Interaction of the renal amylin and renin-angiotensin systems in animal models of diabetes and hypertension. *Miner Electrolyte Metab* 1998; **24**: 389-399 [PMID: 9930378 DOI: 10.1159/000057400]
- 11 **Cooper GJ**, Day AJ, Willis AC, Roberts AN, Reid KB, Leighton B. Amylin and the amylin gene: structure, function and relationship to islet amyloid and to diabetes mellitus. *Biochim Biophys Acta* 1989; **1014**: 247-258 [PMID: 2690958 DOI: 10.1016/0167-4889(89)90220-6]
- 12 **German MS**, Moss LG, Wang J, Rutter WJ. The insulin and islet amyloid polypeptide genes contain similar cell-specific promoter elements that bind identical beta-cell nuclear complexes. *Mol Cell Biol* 1992; **12**: 1777-1788 [PMID: 1549125 DOI: 10.1128/MCB.12.4.1777]
- 13 **Mulder H**, Åhrén B, Sundler F. Islet amyloid polypeptide and insulin gene expression are regulated in parallel by glucose in vivo in rats. *Am J Physiol* 1996; **271**: E1008-E1014 [PMID: 8997219]
- 14 **James JH**, Wagner KR, King JK, Leffler RE, Upputuri RK, Balasubramaniam A, Friend LA, Shelly DA, Paul RJ, Fischer JE. Stimulation of both aerobic glycolysis and Na(+)-K(+)-ATPase activity in skeletal muscle by epinephrine or amylin. *Am J Physiol* 1999; **277**: E176-E186 [PMID: 10409142]
- 15 **Young AA**, Mott DM, Stone K, Cooper GJ. Amylin activates glycogen phosphorylase in the isolated soleus muscle of the rat. *FEBS Lett* 1991; **281**: 149-151 [PMID: 2015885 DOI: 10.1016/0014-5793(91)80380-L]
- 16 **Baumont K**, Kenney MA, Young AA, Rink TJ. High affinity amylin binding sites in rat brain. *Mol Pharmacol* 1993; **44**: 493-497 [PMID: 8396712]
- 17 **Grabauskas G**, Zhou SY, Das S, Lu Y, Owyang C, Moises HC. Prolactin-releasing peptide affects gastric motor function in rat by modulating synaptic transmission in the dorsal vagal complex. *J Physiol* 2004; **561**: 821-839 [PMID: 15486017 DOI: 10.1113/jphysiol.2004.072736]
- 18 **Samsom M**, Szarka LA, Camilleri M, Vella A, Zinsmeister AR, Rizza RA. Pramlintide, an amylin analog, selectively delays gastric emptying: potential role of vagal inhibition. *Am J Physiol Gastrointest Liver Physiol* 2000; **278**: G946-G951 [PMID: 10859225]
- 19 **Woerle HJ**, Albrecht M, Linke R, Zschau S, Neumann C, Nicolaus M, Gerich JE, Göke B, Schirra J. Impaired hyperglycemia-induced delay in gastric emptying in patients with type 1 diabetes deficient for islet amyloid polypeptide. *Diabetes Care* 2008; **31**: 2325-2331 [PMID: 19033417 DOI: 10.2337/dc07-2446]
- 20 **Paulsson JF**, Ludvigsson J, Carlsson A, Casas R, Forsander G, Ivarsson SA, Kockum I, Lernmark Å, Marcus C, Lindblad B, Westermark GT. High plasma levels of islet amyloid polypeptide in young with new-onset of type 1 diabetes mellitus. *PLoS One* 2014; **9**: e93053 [PMID: 24671002 DOI: 10.1371/journal.pone.0093053]
- 21 **Young A**. Inhibition of gastric emptying. *Adv Pharmacol* 2005; **52**: 99-121 [PMID: 16492543 DOI: 10.1016/S1054-3589(05)52006-4]
- 22 **Zhao HL**, Lai FM, Tong PC, Zhong DR, Yang D, Tomlinson B, Chan JC. Prevalence and clinicopathological characteristics of islet amyloid in chinese patients with type 2 diabetes. *Diabetes* 2003; **52**: 2759-2766 [PMID: 14578294 DOI: 10.2337/diabetes.52.11.2759]
- 23 **Kautzky-Willer A**, Thomaseth K, Ludvik B, Nowotny P, Rabensteiner D, Waldhäusl W, Pacini G, Prager R. Elevated islet amyloid pancreatic polypeptide and proinsulin in lean gestational diabetes. *Diabetes* 1997; **46**: 607-614 [PMID: 9075800 DOI: 10.2337/diab.46.4.607]
- 24 **Thomaseth K**, Pacini G, Clodi M, Kautzky-Willer A, Nolan JJ, Prager R, Olefsky JM, Ludvik B. Amylin release during oral glucose tolerance test. *Diabet Med* 1997; **14** Suppl 2: S29-S34 [PMID: 9212327 DOI: 10.1002/(SICI)1096-9136(199706)14:2+<S29::AID-DIA401>3.3.CO;2-S]
- 25 **Thomaseth K**, Kautzky-Willer A, Ludvik B, Prager R, Pacini G. Integrated mathematical model to assess beta-cell activity during the oral glucose test. *Am J Physiol* 1996; **270**: E522-E531 [PMID: 8638701]
- 26 **Ludvik B**, Clodi M, Kautzky-Willer A, Schuller M, Graf H,

- Hartter E, Pacini G, Prager R. Increased levels of circulating islet amyloid polypeptide in patients with chronic renal failure have no effect on insulin secretion. *J Clin Invest* 1994; **94**: 2045-2050 [PMID: 7962550 DOI: 10.1172/JCI117558]
- 27 **de Koning EJ**, van den Brand JJ, Mott VL, Chargé SB, Hansen BC, Bodkin NL, Morris JF, Clark A. Macrophages and pancreatic islet amyloidosis. *Amyloid* 1998; **5**: 247-254 [PMID: 10036582 DOI: 10.3109/13506129809007297]
- 28 **Sanke T**, Bell GI, Sample C, Rubenstein AH, Steiner DF. An islet amyloid peptide is derived from an 89-amino acid precursor by proteolytic processing. *J Biol Chem* 1988; **263**: 17243-17246 [PMID: 3053705]
- 29 **Percy AJ**, Trainor DA, Rittenhouse J, Phelps J, Koda JE. Development of sensitive immunoassays to detect amylin and amylin-like peptides in unextracted plasma. *Clin Chem* 1996; **42**: 576-585 [PMID: 8605675]
- 30 **Butler PC**, Chou J, Carter WB, Wang YN, Bu BH, Chang D, Chang JK, Rizza RA. Effects of meal ingestion on plasma amylin concentration in NIDDM and nondiabetic humans. *Diabetes* 1990; **39**: 752-756 [PMID: 2189768 DOI: 10.2337/diab.39.6.752]
- 31 **Sanke T**, Hanabusa T, Nakano Y, Oki C, Okai K, Nishimura S, Kondo M, Nanjo K. Plasma islet amyloid polypeptide (Amylin) levels and their responses to oral glucose in type 2 (non-insulin-dependent) diabetic patients. *Diabetologia* 1991; **34**: 129-132 [PMID: 2065848 DOI: 10.1007/BF00500385]
- 32 **Koda JE**, Fineman MS, Kolterman OG, Caro JF. 24 hour plasma amylin profiles are elevated in IGT subjects vs. normal controls. *Diabetes* 1995; **44** (suppl 1): 238A
- 33 **Dimisdale JE**, Kolterman O, Koda J, Nelesen R. Effect of race and hypertension on plasma amylin concentrations. *Hypertension* 1996; **27**: 1273-1276 [PMID: 8641735 DOI: 10.1161/01.HYP.27.6.1273]
- 34 **Juhl CB**, Pørksen N, Sturis J, Hansen AP, Veldhuis JD, Pincus S, Fineman M, Schmitz O. High-frequency oscillations in circulating amylin concentrations in healthy humans. *Am J Physiol Endocrinol Metab* 2000; **278**: E484-E490 [PMID: 10710503]
- 35 **Blackard WG**, Clore JN, Kellum JM. Amylin/insulin secretory ratios in morbidly obese man: inverse relationship with glucose disappearance rate. *J Clin Endocrinol Metab* 1994; **78**: 1257-1260 [PMID: 8175987]
- 36 **Gulli G**, Rossetti L, DeFronzo RA. Hyperamylinemia is associated with hyperinsulinemia in the glucose-tolerant, insulin-resistant offspring of two Mexican-American non-insulin-dependent diabetic parents. *Metabolism* 1997; **46**: 1157-1161 [PMID: 9322799 DOI: 10.1016/S0026-0495(97)90209-2]
- 37 **Kailasam MT**, Parmer RJ, Tyrell EA, Henry RR, O'Connor DT. Circulating amylin in human essential hypertension: heritability and early increase in individuals at genetic risk. *J Hypertens* 2000; **18**: 1611-1620 [PMID: 11081774 DOI: 10.1097/00004872-200018110-00012]
- 38 **Koda JE**, Fineman M, Rink TJ, Dailey GE, Muchmore DB, Linarelli LG. Amylin concentrations and glucose control. *Lancet* 1992; **339**: 1179-1180 [PMID: 1349407 DOI: 10.1016/0140-6736(92)90785-2]
- 39 **Gasa R**, Gomis R, Casamitjana R, Novials A. High glucose concentration favors the selective secretion of islet amyloid polypeptide through a constitutive secretory pathway in human pancreatic islets. *Pancreas* 2001; **22**: 307-310 [PMID: 11291934 DOI: 10.1097/00006676-200104000-00013]
- 40 **Hou X**, Ling Z, Quartier E, Foriers A, Schuit F, Pipeleers D, Van Schravendijk C. Prolonged exposure of pancreatic beta cells to raised glucose concentrations results in increased cellular content of islet amyloid polypeptide precursors. *Diabetologia* 1999; **42**: 188-194 [PMID: 10064099 DOI: 10.1007/s001250051138]
- 41 **Rochet JC**, Lansbury PT. Amyloid fibrillogenesis: themes and variations. *Curr Opin Struct Biol* 2000; **10**: 60-68 [PMID: 10679462 DOI: 10.1016/S0959-440X(99)00049-4]
- 42 **Höppener JW**, Oosterwijk C, van Hulst KL, Verbeek JS, Capel PJ, de Koning EJ, Clark A, Jansz HS, Lips CJ. Molecular physiology of the islet amyloid polypeptide (IAPP)/amylin gene in man, rat, and transgenic mice. *J Cell Biochem* 1994; **55** Suppl: 39-53 [PMID: 7929617 DOI: 10.1002/jcb.240550006]
- 43 **Westermarck P**, Benson MD, Buxbaum JN, Cohen AS, Frangione B, Ikeda S, Masters CL, Merlini G, Saraiva MJ, Sipe JD. A primer of amyloid nomenclature. *Amyloid* 2007; **14**: 179-183 [PMID: 17701465 DOI: 10.1080/13506120701460923]
- 44 **Westermarck P**. Aspects on human amyloid forms and their fibril polypeptides. *FEBS J* 2005; **272**: 5942-5949 [PMID: 16302959 DOI: 10.1111/j.1742-4658.2005.05024.x]
- 45 **Yao Y**, Wang SX, Zhang YK, Qu Z, Liu G, Zou WZ. A clinicopathological analysis in a large cohort of Chinese patients with renal amyloid light-chain amyloidosis. *Nephrol Dial Transplant* 2013; **28**: 689-697 [PMID: 23182813 DOI: 10.1093/ndt/gfs501]
- 46 **Kojro E**, Postina R. Regulated proteolysis of RAGE and AbetaPP as possible link between type 2 diabetes mellitus and Alzheimer's disease. *J Alzheimers Dis* 2009; **16**: 865-878 [PMID: 19387119 DOI: 10.3233/JAD-2009-0998]
- 47 **Srodulski S**, Sharma S, Bachstetter AB, Brelsfoard JM, Pascual C, Xie XS, Saatman KE, Van Eldik LJ, Despa F. Neuroinflammation and neurologic deficits in diabetes linked to brain accumulation of amylin. *Mol Neurodegener* 2014; **9**: 30 [PMID: 25149184 DOI: 10.1186/1750-1326-9-30 DOI: 10.1186/1750-1326-9-30]
- 48 **Sakagashira S**, Hiddinga HJ, Tateishi K, Sanke T, Hanabusa T, Nanjo K, Eberhardt NL. S20G mutant amylin exhibits increased in vitro amyloidogenicity and increased intracellular cytotoxicity compared to wild-type amylin. *Am J Pathol* 2000; **157**: 2101-2109 [PMID: 11106582 DOI: 10.1016/S0002-9440(10)64848-1]
- 49 **Sakagashira S**, Sanke T, Hanabusa T, Shimomura H, Ohagi S, Kumagaye KY, Nakajima K, Nanjo K. Missense mutation of amylin gene (S20G) in Japanese NIDDM patients. *Diabetes* 1996; **45**: 1279-1281 [PMID: 8772735 DOI: 10.2337/diab.45.9.1279]
- 50 **Seino S**. S20G mutation of the amylin gene is associated with Type II diabetes in Japanese. Study Group of Comprehensive Analysis of Genetic Factors in Diabetes Mellitus. *Diabetologia* 2001; **44**: 906-909 [PMID: 11508277 DOI: 10.1007/s001250100531]
- 51 **Yamada K**, Yuan X, Ishiyama S, Nonaka K. Glucose tolerance in Japanese subjects with S20G mutation of the amylin gene. *Diabetologia* 1998; **41**: 125 [PMID: 9498642 DOI: 10.1007/s001250050878]
- 52 **Lee SC**, Hashim Y, Li JK, Ko GT, Critchley JA, Cockram CS, Chan JC. The islet amyloid polypeptide (amylin) gene S20G mutation in Chinese subjects: evidence for associations with type 2 diabetes and cholesterol levels. *Clin Endocrinol (Oxf)* 2001; **54**: 541-546 [PMID: 11318791 DOI: 10.1046/j.1365-2265.2001.01244.x]
- 53 **Ng MC**, Lee SC, Ko GT, Li JK, So WY, Hashim Y, Barnett AH, Mackay IR, Critchley JA, Cockram CS, Chan JC. Familial early-onset type 2 diabetes in Chinese patients: obesity and genetics have more significant roles than autoimmunity. *Diabetes Care* 2001; **24**: 663-671 [PMID: 11315828 DOI: 10.2337/diacare.24.4.663]
- 54 **Chuang LM**, Lee KC, Huang CN, Wu HP, Tai TY, Lin BJ. Role of S20G mutation of amylin gene in insulin secretion, insulin sensitivity, and type II diabetes mellitus in Taiwanese patients. *Diabetologia* 1998; **41**: 1250-1251 [PMID: 9794116 DOI: 10.1007/s001250051060]
- 55 **Cook JT**, Patel PP, Clark A, Höppener JW, Lips CJ, Mosselman S, O'Rahilly S, Page RC, Wainscoat JS, Turner RC. Non-linkage of the islet amyloid polypeptide gene with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 1991; **34**: 103-108 [PMID: 1676684 DOI: 10.1007/BF00500380]
- 56 **Peila R**, Rodriguez BL, Launer LJ. Type 2 diabetes, APOE gene, and the risk for dementia and related pathologies: The Honolulu Asia Aging Study. *Diabetes* 2002; **51**: 1256-1262 [PMID: 11916953 DOI: 10.2337/diabetes.51.4.1256]
- 57 **Guan J**, Zhao HL, Sui Y, He L, Lee HM, Lai FM, Tong PC, Chan JC. Histopathological correlations of islet amyloidosis with apolipoprotein E polymorphisms in type 2 diabetic Chinese patients. *Pancreas* 2013; **42**: 1129-1137 [PMID: 24005233 DOI: 10.1097/MPA.0b013e3182965e6e]
- 58 **Chien V**, Aitken JF, Zhang S, Buchanan CM, Hickey A, Brittain T, Cooper GJ, Loomes KM. The chaperone proteins HSP70,

- HSP40/DnaJ and GRP78/BiP suppress misfolding and formation of  $\beta$ -sheet-containing aggregates by human amylin: a potential role for defective chaperone biology in Type 2 diabetes. *Biochem J* 2010; **432**: 113-121 [PMID: 20735358 DOI: 10.1042/BJ20100434]
- 59 **Peuralinna T**, Tanskanen M, Mäkelä M, Polvikoski T, Paetau A, Kalimo H, Sulkava R, Hardy J, Lai SL, Arepalli S, Hernandez D, Traynor BJ, Singleton A, Tienari PJ, Myllykangas L. APOE and A $\beta$ PP gene variation in cortical and cerebrovascular amyloid- $\beta$  pathology and Alzheimer's disease: a population-based analysis. *J Alzheimers Dis* 2011; **26**: 377-385 [PMID: 21654062 DOI: 10.3233/JAD-2011-102049]
  - 60 **Veereshwarayya V**, Kumar P, Rosen KM, Mestrlil R, Querfurth HW. Differential effects of mitochondrial heat shock protein 60 and related molecular chaperones to prevent intracellular beta-amyloid-induced inhibition of complex IV and limit apoptosis. *J Biol Chem* 2006; **281**: 29468-29478 [PMID: 16887805 DOI: 10.1074/jbc.M602533200]
  - 61 **Yip AG**, McKee AC, Green RC, Wells J, Young H, Cupples LA, Farrer LA. APOE, vascular pathology, and the AD brain. *Neurology* 2005; **65**: 259-265 [PMID: 16043796 DOI: 10.1212/01.wnl.0000168863.49053.4d]
  - 62 **Mahley RW**, Rall SC. Apolipoprotein E: far more than a lipid transport protein. *Annu Rev Genomics Hum Genet* 2000; **1**: 507-537 [PMID: 11701639 DOI: 10.1146/annurev.genom.1.1.507]
  - 63 **Hickey AJ**, Bradley JW, Skea GL, Middleditch MJ, Buchanan CM, Phillips AR, Cooper GJ. Proteins associated with immunopurified granules from a model pancreatic islet beta-cell system: proteomic snapshot of an endocrine secretory granule. *J Proteome Res* 2009; **8**: 178-186 [PMID: 19055480 DOI: 10.1021/pr800675k]
  - 64 **Magrané J**, Smith RC, Walsh K, Querfurth HW. Heat shock protein 70 participates in the neuroprotective response to intracellularly expressed beta-amyloid in neurons. *J Neurosci* 2004; **24**: 1700-1706 [PMID: 14973234 DOI: 10.1523/JNEUROSCI.4330-03.2004]
  - 65 **Zhang M**, Hu R, Chen H, Chang Y, Ma J, Liang G, Mi J, Wang Y, Zheng J. Polymorphic cross-seeding amyloid assemblies of amyloid- $\beta$  and human islet amyloid polypeptide. *Phys Chem Chem Phys* 2015; **17**: 23245-23256 [PMID: 26283068 DOI: 10.1039/c5cp03329b]
  - 66 **O'Nuallain B**, Williams AD, Westermark P, Wetzel R. Seeding specificity in amyloid growth induced by heterologous fibrils. *J Biol Chem* 2004; **279**: 17490-17499 [PMID: 14752113 DOI: 10.1074/jbc.M311300200]
  - 67 **Hartley DM**, Walsh DM, Ye CP, Diehl T, Vasquez S, Vassilev PM, Teplow DB, Selkoe DJ. Protofibrillar intermediates of amyloid beta-protein induce acute electrophysiological changes and progressive neurotoxicity in cortical neurons. *J Neurosci* 1999; **19**: 8876-8884 [PMID: 10516307]
  - 68 **Maloy AL**, Longnecker DS, Greenberg ER. The relation of islet amyloid to the clinical type of diabetes. *Hum Pathol* 1981; **12**: 917-922 [PMID: 7028600]
  - 69 **Bukau B**, Weissman J, Horwich A. Molecular chaperones and protein quality control. *Cell* 2006; **125**: 443-451 [PMID: 16678092 DOI: 10.1016/j.cell.2006.04.014]
  - 70 **Chargé SB**, de Koning EJ, Clark A. Effect of pH and insulin on fibrillogenesis of islet amyloid polypeptide in vitro. *Biochemistry* 1995; **34**: 14588-14593 [PMID: 7578065]
  - 71 **Dobson CM**. Principles of protein folding, misfolding and aggregation. *Semin Cell Dev Biol* 2004; **15**: 3-16 [PMID: 15036202 DOI: 10.1016/j.semdb.2003.12.008]
  - 72 **Westermark P**, Engström U, Johnson KH, Westermark GT, Betsholtz C. Islet amyloid polypeptide: pinpointing amino acid residues linked to amyloid fibril formation. *Proc Natl Acad Sci USA* 1990; **87**: 5036-5040 [PMID: 2195544]
  - 73 **Betsholtz C**, Christmansson L, Engström U, Rorsman F, Jordan K, O'Brien TD, Murtaugh M, Johnson KH, Westermark P. Structure of cat islet amyloid polypeptide and identification of amino acid residues of potential significance for islet amyloid formation. *Diabetes* 1990; **39**: 118-122 [PMID: 2210054]
  - 74 **Lorenzo A**, Razzaboni B, Weir GC, Yankner BA. Pancreatic islet cell toxicity of amylin associated with type-2 diabetes mellitus. *Nature* 1994; **368**: 756-760 [PMID: 8152488]
  - 75 **Janciauskiene S**, Åhrén B. Fibrillar islet amyloid polypeptide differentially affects oxidative mechanisms and lipoprotein uptake in correlation with cytotoxicity in two insulin-producing cell lines. *Biochem Biophys Res Commun* 2000; **267**: 619-625 [PMID: 10631112 DOI: 10.1006/bbrc.1999.1989]
  - 76 **Hardy J**, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* 2002; **297**: 353-356 [PMID: 12130773 DOI: 10.1126/science.1072994]
  - 77 **Zhao HL**, Sui Y, Guan J, He L, Gu XM, Wong HK, Baum L, Lai FM, Tong PC, Chan JC. Amyloid oligomers in diabetic and nondiabetic human pancreas. *Transl Res* 2009; **153**: 24-32 [PMID: 19100955 DOI: 10.1016/j.trsl.2008.10.009]
  - 78 **Li XL**, Chen T, Wong YS, Xu G, Fan RR, Zhao HL, Chan JC. Involvement of mitochondrial dysfunction in human islet amyloid polypeptide-induced apoptosis in INS-1E pancreatic beta cells: An effect attenuated by phycocyanin. *Int J Biochem Cell Biol* 2011; **43**: 525-534 [PMID: 21163363 DOI: 10.1016/j.biocel.2010.12.008]
  - 79 **Kayed R**, Head E, Thompson JR, McIntire TM, Milton SC, Cotman CW, Glabe CG. Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. *Science* 2003; **300**: 486-489 [PMID: 12702875 DOI: 10.1126/science.1079469]
  - 80 **Tomic JL**, Pensalfini A, Head E, Glabe CG. Soluble fibrillar oligomer levels are elevated in Alzheimer's disease brain and correlate with cognitive dysfunction. *Neurobiol Dis* 2009; **35**: 352-358 [PMID: 19523517 DOI: 10.1016/j.nbd.2009.05.024]
  - 81 **Zraika S**, Hull RL, Verchere CB, Clark A, Potter KJ, Fraser PE, Raleigh DP, Kahn SE. Toxic oligomers and islet beta cell death: guilty by association or convicted by circumstantial evidence? *Diabetologia* 2010; **53**: 1046-1056 [PMID: 20182863 DOI: 10.1007/s00125-010-1671-6]
  - 82 **Gurlo T**, Ryazantsev S, Huang CJ, Yeh MW, Reber HA, Hines OJ, O'Brien TD, Glabe CG, Butler PC. Evidence for proteotoxicity in beta cells in type 2 diabetes: toxic islet amyloid polypeptide oligomers form intracellularly in the secretory pathway. *Am J Pathol* 2010; **176**: 861-869 [PMID: 20042670 DOI: 10.2353/ajpath.2010.090532]
  - 83 **Pittner RA**, Albrandt K, Beaumont K, Gaeta LS, Koda JE, Moore CX, Rittenhouse J, Rink TJ. Molecular physiology of amylin. *J Cell Biochem* 1994; **55** Suppl: 19-28 [PMID: 7929615]
  - 84 **Fineman MS**, Koda JE, Shen LZ, Strobel SA, Maggs DG, Weyer C, Kolterman OG. The human amylin analog, pramlintide, corrects postprandial hyperglucagonemia in patients with type 1 diabetes. *Metabolism* 2002; **51**: 636-641 [PMID: 11979398]
  - 85 **Fineman M**, Weyer C, Maggs DG, Strobel S, Kolterman OG. The human amylin analog, pramlintide, reduces postprandial hyperglucagonemia in patients with type 2 diabetes mellitus. *Horm Metab Res* 2002; **34**: 504-508 [PMID: 12384827 DOI: 10.1055/s-2002-34790]
  - 86 **Herrmann K**, Frias JP, Edelman SV, Lutz K, Shan K, Chen S, Maggs D, Kolterman OG. Pramlintide improved measures of glycemic control and body weight in patients with type 1 diabetes mellitus undergoing continuous subcutaneous insulin infusion therapy. *Postgrad Med* 2013; **125**: 136-144 [PMID: 23748514 DOI: 10.3810/pgm.2013.05.2635]
  - 87 **Weinzimer SA**, Sherr JL, Cengiz E, Kim G, Ruiz JL, Carria L, Voskanyan G, Roy A, Tamborlane WV. Effect of pramlintide on prandial glycemic excursions during closed-loop control in adolescents and young adults with type 1 diabetes. *Diabetes Care* 2012; **35**: 1994-1999 [PMID: 22815298 DOI: 10.2337/dc12-0330]
  - 88 **Maggs DG**, Fineman M, Kornstein J, Burrell T, Schwartz S, Wang Y, Ruggles JA, Kolterman OG, Weyer C. Pramlintide reduces postprandial glucose excursions when added to insulin lispro in subjects with type 2 diabetes: a dose-timing study. *Diabetes Metab Res Rev* 2004; **20**: 55-60 [PMID: 14737746 DOI: 10.1002/dmrr.419]
  - 89 **Weyer C**, Gottlieb A, Kim DD, Lutz K, Schwartz S, Gutierrez M, Wang Y, Ruggles JA, Kolterman OG, Maggs DG. Pramlintide reduces postprandial glucose excursions when added to regular insulin or insulin lispro in subjects with type 1 diabetes: a dose-

- timing study. *Diabetes Care* 2003; **26**: 3074-3079 [PMID: 14578242]
- 90 **Ratner RE**, Dickey R, Fineman M, Maggs DG, Shen L, Strobel SA, Weyer C, Kolterman OG. Amylin replacement with pramlintide as an adjunct to insulin therapy improves long-term glycaemic and weight control in Type 1 diabetes mellitus: a 1-year, randomized controlled trial. *Diabet Med* 2004; **21**: 1204-1212 [PMID: 15498087 DOI: 10.1111/j.1464-5491.2004.01319.x]
  - 91 **Whitehouse F**, Kruger DF, Fineman M, Shen L, Ruggles JA, Maggs DG, Weyer C, Kolterman OG. A randomized study and open-label extension evaluating the long-term efficacy of pramlintide as an adjunct to insulin therapy in type 1 diabetes. *Diabetes Care* 2002; **25**: 724-730 [PMID: 11919132]
  - 92 **Edelman S**, Garg S, Frias J, Maggs D, Wang Y, Zhang B, Strobel S, Lutz K, Kolterman O. A double-blind, placebo-controlled trial assessing pramlintide treatment in the setting of intensive insulin therapy in type 1 diabetes. *Diabetes Care* 2006; **29**: 2189-2195 [PMID: 17003291 DOI: 10.2337/dc06-0042]
  - 93 **Ratner RE**, Want LL, Fineman MS, Velte MJ, Ruggles JA, Gottlieb A, Weyer C, Kolterman OG. Adjunctive therapy with the amylin analogue pramlintide leads to a combined improvement in glycemic and weight control in insulin-treated subjects with type 2 diabetes. *Diabetes Technol Ther* 2002; **4**: 51-61 [PMID: 12017421 DOI: 10.1089/15209150252924094]
  - 94 **Hollander PA**, Levy P, Fineman MS, Maggs DG, Shen LZ, Strobel SA, Weyer C, Kolterman OG. Pramlintide as an adjunct to insulin therapy improves long-term glycemic and weight control in patients with type 2 diabetes: a 1-year randomized controlled trial. *Diabetes Care* 2003; **26**: 784-790 [PMID: 12610038]
  - 95 **Riddle M**, Frias J, Zhang B, Maier H, Brown C, Lutz K, Kolterman O. Pramlintide improved glycemic control and reduced weight in patients with type 2 diabetes using basal insulin. *Diabetes Care* 2007; **30**: 2794-2799 [PMID: 17698615 DOI: 10.2337/dc07-0589]
  - 96 **Thompson RG**, Gottlieb A, Organ K, Koda J, Kisicki J, Kolterman OG. Pramlintide: a human amylin analogue reduced postprandial plasma glucose, insulin, and C-peptide concentrations in patients with type 2 diabetes. *Diabet Med* 1997; **14**: 547-555 [PMID: 9223392]
  - 97 **Treusch S**, Cyr DM, Lindquist S. Amyloid deposits: protection against toxic protein species? *Cell Cycle* 2009; **8**: 1668-1674 [PMID: 19411847]
  - 98 **Aitken JF**, Loomes KM, Konarkowska B, Cooper GJ. Suppression by polycyclic compounds of the conversion of human amylin into insoluble amyloid. *Biochem J* 2003; **374**: 779-784 [PMID: 12812521 DOI: 10.1042/BJ20030422]
  - 99 **Olcott AP**, Tian J, Walker V, Dang H, Middleton B, Adorini L, Washburn L, Kaufman DL. Antigen-based therapies using ignored determinants of beta cell antigens can more effectively inhibit late-stage autoimmune disease in diabetes-prone mice. *J Immunol* 2005; **175**: 1991-1999 [PMID: 16034144]
  - 100 **Westwell-Roper C**, Dunne A, Kim ML, Verchere CB, Masters SL. Activating the NLRP3 inflammasome using the amyloidogenic peptide IAPP. *Methods Mol Biol* 2013; **1040**: 9-18 [PMID: 23852593 DOI: 10.1007/978-1-62703-523-1\_2]
  - 101 **Baker RL**, Delong T, Barbour G, Bradley B, Nakayama M, Haskins K. Cutting edge: CD4 T cells reactive to an islet amyloid polypeptide peptide accumulate in the pancreas and contribute to disease pathogenesis in nonobese diabetic mice. *J Immunol* 2013; **191**: 3990-3994 [PMID: 24043895 DOI: 10.4049/jimmunol.1301480]
  - 102 **Zhang XX**, Shen J, Liao QY, Li R, Zhang QJ, He L, Zhao HL. Human Amylin Induces CD4 CD25 Foxp3 Regulatory T Cells in the Protection from Autoimmune Diabetes [abstract]. *Diabetes* 2015; **64** (suppl 1): 1804-P

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## Insulin-secreting $\beta$ cells require a post-genomic concept

Fang-Xu Jiang, Grant Morahan

Fang-Xu Jiang, Islet Cell Development Program, Harry Perkins Institute of Medical Research, Nedlands, WA 6009, Australia

Grant Morahan, Centre for Diabetes Research, the University of Western Australia, Perth, WA 6009, Australia

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Correspondence to: Dr. Fang-Xu Jiang, Islet Cell Development Program, Harry Perkins Institute of Medical Research, 6 Verdun St, Nedlands, WA 6009, Australia. [fang-xu.jiang@perkins.uwa.edu.au](mailto:fang-xu.jiang@perkins.uwa.edu.au)  
Telephone: +61-8-61510758  
Fax: +61-8-61510701

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### Abstract

Pancreatic insulin-secreting  $\beta$  cells are essential in maintaining normal glucose homeostasis accomplished by

highly specialized transcription of insulin gene, of which occupies up to 40% their transcriptome. Deficiency of these cells causes diabetes mellitus, a global public health problem. Although tremendous endeavors have been made to generate insulin-secreting cells from human pluripotent stem cells (*i.e.*, primitive cells capable of giving rise to all cell types in the body), a regenerative therapy to diabetes has not yet been established. Furthermore, the nomenclature of  $\beta$  cells has become inconsistent, confusing and controversial due to the lack of standardized positive controls of developmental stage-matched *in vivo* cells. In order to minimize this negative impact and facilitate critical research in this field, a post-genomic concept of pancreatic  $\beta$  cells might be helpful. In this review article, we will briefly describe how  $\beta$  cells were discovered and islet lineage is developed that may help understand the cause of nomenclatural controversy, suggest a post-genomic definition and finally provide a conclusive remark on future research of this pivotal cell.

**Key words:** Beta cells; Insulin; Post-genome; Concept

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**Core tip:** Pancreatic  $\beta$  cells are highly effective and efficient in the production of insulin, and specialized in its regulated secretion. Deficiency of  $\beta$  cells causes diabetes mellitus, the prevalence of which keeps climbing, despite new drugs continuously becoming available to clinics. Thus regenerative therapies to this devastating disease show great promise. Nevertheless, the generation of  $\beta$  cells requires multiple forced fate changes from pluripotent stem cells and the latter derived insulin+ cells expressing selective key  $\beta$ -cell transcription factors may not be the genuine islet counterparts. Hence their post-genomic concept may help the future development of diabetes regenerative therapies.

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## INTRODUCTION

Pancreatic insulin-secreting  $\beta$  cells are of pivotal importance to our physiology because they play a central role in maintaining normal glucose homeostasis by their ability to produce and secrete insulin - a life hormone released in a fine-tuned manner as the body requires it. Deficiency of glucose-responsive  $\beta$  cells causes diabetes mellitus, a global public health issue with a progressively increasing prevalence. Absolute deficiency of these  $\beta$  cells due to autoimmune-mediated destruction results in type 1 diabetes mellitus (T1D). Relative deficiency leads to type 2 diabetes mellitus (T2D), caused by multiple issues, such as the failure of peripheral metabolic tissues to respond to insulin action, the liver's inability to control the production of glucose and the demise of islet  $\beta$  cells<sup>[1]</sup>. Diabetes mellitus currently affects over 387 million people worldwide. Despite a variety of treatments being continuously brought to the clinic, the incidence of this disorder is progressively climbing and is projected to reach 592 million by 2035<sup>[2]</sup>. Thus, there is an urgent need for novel treatments, such as regenerative medicine, a field established by the creation of human embryonic stem cells (ESCs) in 1998<sup>[3]</sup>. A regenerative therapy would provide a cure of T1D (should autoimmunity to  $\beta$  cells be controlled) and also for a subset of T2D, either by transplantation of donated hormone-secreting islets<sup>[4]</sup> or of *in vitro* generated genuine  $\beta$  cells from ESCs or induced pluripotent stem cells (iPSCs), or ultimately by regeneration *in situ* of endogenous  $\beta$  cells.

For diabetes regenerative medicine, tremendous focus has been applied to generate insulin-secreting  $\beta$  cells *in vitro*. However, over the years the nomenclature of  $\beta$  cells has unfortunately become inconsistent, confused and controversial, which in turn has apparently hampered the progress of the field. In order to minimize the negative impact of this confusion and to facilitate critical research, we suggest a post-genomic concept of pancreatic  $\beta$  cells. We will briefly describe how  $\beta$  cells were discovered and the islet lineage developed; how this controversy arose; suggest a post-genomic definition and finally provide concluding remarks on this vital research.

## BRIEF HISTORICAL ACCOUNT

The "islet of Langerhans" was named after Paul Langerhans, a German medical student, who in 1869 observed small clusters of "clear cells" within the pancreas that were obviously different from the surrounding pancreatic tissue. Subsequently, Edouard Laguesse termed these clusters as islets of Langerhans (Figure 1). Approximately 30 years later, in 1907, Falkmer *et al.*<sup>[5]</sup> found the islet cells harbored distinct granules that were different from the zymogen granules in the acinar cells. For example, one type of islet cells was basophilic (type B) stained by

certain histochemical methods and another was not (type A). A more detailed description of how different types of endocrine cells in the pancreas may be distinguished is documented elsewhere.

In the year 1922, the B cells were discovered to produce the hormone insulin (Figure 1) by Banting and Best<sup>[6]</sup> who were awarded the Nobel prize for Medicine in 1923. The presence of insulin in the B cells (now known as  $\beta$  cells) was first confirmed immunohistochemically in 1957<sup>[7]</sup>. Glucagon was identified in the A cells (now known as  $\alpha$  cells) in 1962; this hormone raises blood sugar levels by releasing glucose stored in the liver as glycogen, which is formed in a process called gluconeogenesis. Insulin was the first protein to be fully sequenced (*cf.* Figure 1). This was accomplished by Frederick Sanger's group in 1955<sup>[8]</sup> and in 1958 Sanger received the Nobel Prize in Chemistry for this landmark discovery. In 1977, Ullrich *et al.*<sup>[9]</sup> successfully cloned the insulin gene and its cDNA using recombinant DNA technology.

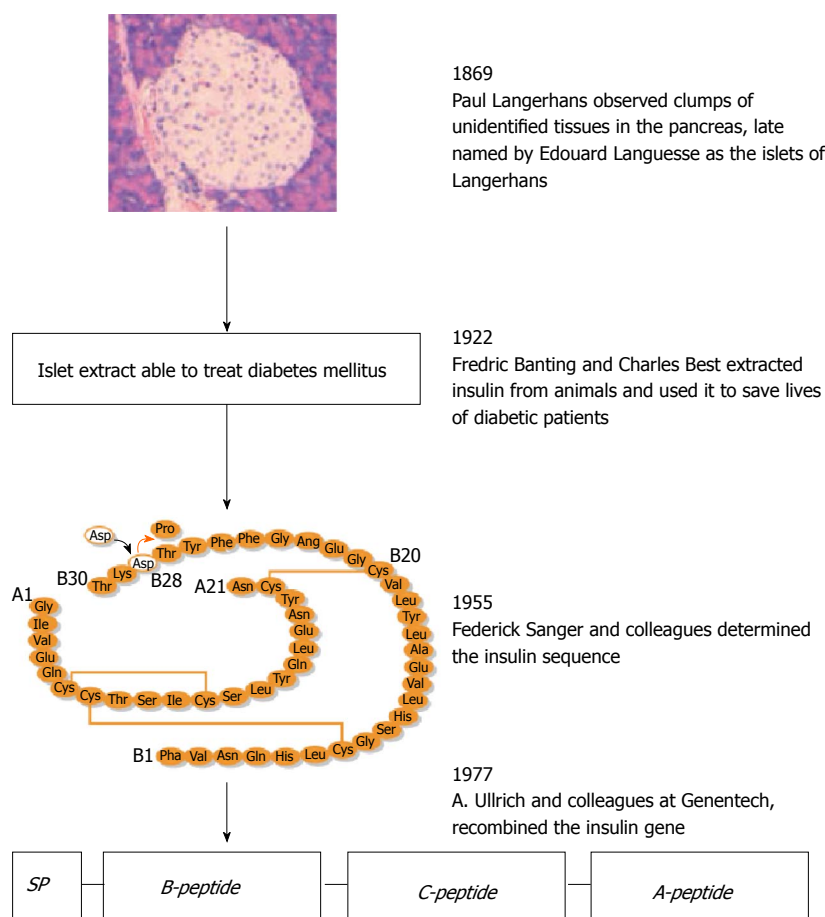
Since then, knowledge of this important cell type has increased exponentially. In particular following the creation of human ESCs, numerous academic groups and biotechnological companies have attempted to generate  $\beta$  cells *in vitro* from pluripotent stem cells (PSCs, which include ESCs and iPSCs) with the aim of advancing pancreas developmental biology, providing a renewable cell source for drug screening and, ultimately, establishing a regenerative therapy for diabetes. However, an associated negative effect of this period was the appearance of controversies and confusions on the definition of  $\beta$  cells. This confusion arose from simplistically treating PSC-derived insulin<sup>+</sup> cells expressing several markers of key  $\beta$ -cell transcription factors as a genuine counterpart of *in vivo* glucose-responding cells. In order to help understand this complex and controversial issue, we will briefly introduce the embryology of pancreas development.

## EMBRYOLOGY

The pancreas is an endocrine as well as exocrine organ. It is derived from the primitive germ cell layer known as endoderm (the other two layers are the ectoderm and mesoderm) that originates from the inner cell mass from which ESCs were also originally derived. After gastrulation, the thickened endodermal epithelium along the dorsal and ventral surfaces of the posterior foregut gives rise to the primitive pancreas. In mice, this thickening can be identified histologically at embryonic day (E) 9.0-9.5<sup>[10]</sup>.

The columnar epithelial cells expand into adjacent mesoderm-derived mesenchymal tissue and form the dorsal and ventral buds of the pancreas primordia. These expanding and branching buds fuse together as the developing gut rotates. The fused developing pancreas continues to grow, differentiate and, ultimately, develop into the mature organ. The adult pancreas consists of digestive fluid-transporting ductal tissue, digestive enzyme-secreting acinar tissue and hormone-secreting





**Figure 1** Historical account of the discovery of insulin. Presented four landmark discoveries of the islets of Langerhans, insulin, the sequencing of insulin and insulin gene recombinant technology.

endocrine tissue located in the islets of Langerhans. The latter consist of five types of endocrine cells including in addition to the afore-mentioned  $\beta$  cells and  $\alpha$  cells, somatostatin-secreting  $\delta$  cells, pancreatic polypeptide-secreting PP cells and ghrelin-secreting  $\epsilon$  cells.

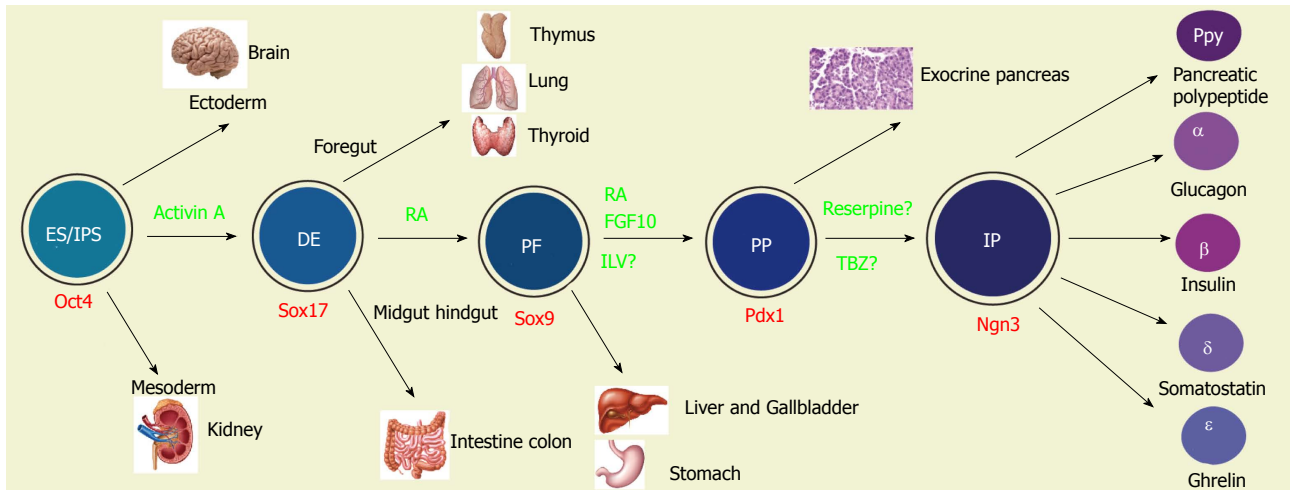
Naturally, human pancreas development displays some features not observed in rodents. For example, the dorsal bud can be detected as early as 26 d post conception (dpc), an equivalent stage to E9.5 embryos in mice, but embryonic  $\beta$  cells are not visible until 52 dpc, approximately 2 wk later than the equivalent stage at which they could be detected in mice. The ontogeny of human embryonic  $\beta$  cells precedes that of embryonic  $\alpha$  cells at 8-10 wk of development<sup>[11]</sup>. Genetic lineage tracing in mice demonstrates that embryonic  $\beta$  cells do not become postnatal functional insulin-secreting  $\beta$  cells<sup>[12]</sup>. All islet cells are detectable at the end of the first trimester in humans<sup>[11]</sup>, but at very later stages (E17.5) in mice<sup>[13]</sup>. These data indicate that the sequence of key developmental events in human pancreatic development is distinct from that in mouse<sup>[14]</sup>, and this is supported by differences in gene expression patterns during both developmental and disease processes in these species<sup>[15]</sup>. Further details of human pancreas development can be found in reviews elsewhere<sup>[16-20]</sup>. In the following sections, we will discuss several intermediate stages of islet development, in order to help understand how the confusing and controversial terminology concerning

insulin-producing  $\beta$  cells appeared.

## DEVELOPMENT OF INSULIN-SECRETING $\beta$ CELLS

### Definitive endoderm

One of three germ layers to appear during embryogenesis, the definitive endoderm gives rise to numerous organs in a process that is summarized in Figure 2. ESCs can be made *in vitro* to recapitulate their *in vivo* developmental pathways, to give rise to definitive endodermal (DE)-like cells by being cultured in the presence of a high concentration of activin A, a member of the transforming growth factor  $\beta$  superfamily. ESC-derived human expandable DE-like cells are termed endodermal progenitors<sup>[21]</sup>. Remarkably, they have been shown to self-renew in the presence of a group of growth factors comprised of bone morphogenetic protein 4, fibroblast growth factor 2, vascular endothelial growth factor and epidermal growth factor<sup>[21]</sup>. These progenitors can be passaged at least 24 times with a population expansion of five orders of magnitude. Furthermore, reprogrammed fibroblast-derived DE-like cells have been independently demonstrated to be capable of expanding approximately 65000-fold in the presence of activin A and LiCl<sup>[22]</sup>. These data suggest that these DE-like cells are highly proliferative. To ensure their correct



**Figure 2 Multiple fate commitments of pluripotent stem cells lead to the development of insulin-secreting  $\beta$  cells.** Whereas inner cell mass (ICM) gives rise to three germ layers (the ectoderm, mesoderm and endoderm) during gastrulation, embryonic stem cells (ESC) or induced pluripotent stem cells (iPSC) preferentially differentiate into definitive endodermal cells [DE, marked by the expression of Sox17 (the Sry-related HMG box transcription factor 17) and Foxa2 (foxhead homeobox 2a)] in the presence of activin A. Along the anterior-posterior axis the DE is divided into foregut (giving rise to the lung, thymus and oesophagus), posterior foregut [PF, marked by the expression of the transcription factor Sox9 and hindgut (committing the intestine and colon)]. *In vitro*, retinoid acid would direct the DE cells to PF cells. Rather than to the stomach, liver and gallbladder, the PF cells preferentially give rise to pancreatic progenitors (PP, marked by the expression of the transcription factor Pdx1) in the presence of retinoid acid (RA) and fibroblast growth factor 10 (FGF10) or indolactam (ILV). Principally towards the exocrine and ductal tissues, the PP also commits to progenitors of the endocrine islet lineages [IP, marked by the expression of a high level of Ngn3, as well as NeuroD1 (neural differentiation 1), IA1 (insulinoma associated 1), Isl1 (Islet 1), Pax6 (paired box factor 6) and Rfx6]. The ES/iPSC-derived Pdx1<sup>+</sup> cells gave rise to Ngn3<sup>+</sup> cells in the presence of tetrabenzine (TBZ). The IP then differentiates into five types of islet cells [ $\alpha$ ,  $\beta$ ,  $\delta$  (somatostatin), PP (pancreatic polypeptide) and  $\epsilon$  (ghrelin)]. The “?” indicates that the differentiation factors have not yet been completely validated.

differentiation, the endodermal progenitors should be transcriptomically compared to isolated embryo derived DE cells, at least with mouse cells. Although further studies are required, these endodermal progenitors may provide expandable pre-pancreas progenitors for generation of insulin-secreting  $\beta$  cells.

### Sox9-expressing progenitors

Sox genes transcribe members of the Sry (sex determining region Y) box-related high-mobility group transcription factor family and are versatile regulators of the stem/progenitor cell fate<sup>[23]</sup> as well as of embryonic development of many organs including the pancreas. Sox9 is a critical transcription factor detectable at E10.5 in the dorsal and ventral pancreatic epithelia<sup>[24]</sup>. Importantly, Sox9-expressing embryonic pancreatic epithelia at E13.5 have the capacity to give rise to acinar, ductal and islet lineages in the pancreas<sup>[25]</sup>. However, Sox9 expression is gradually confined to pancreatic duct cells by E16.5<sup>[25]</sup>. Lineage tracing studies demonstrate that Sox9 is also expressed in other posterior foregut-derived organs including the bile duct, the duodenum and the liver. For example, it is expressed in bile duct cells adjacent to the portal vein from E16.5. Sox9 is also broadly expressed in the intestinal epithelia at E13.5 but become restricted to the crypt from E18.5<sup>[25]</sup>. These data indicate that PSC-derived Sox9-expressing cells may commit to multiple endoderm-derived lineages including the pancreas.

### Pancreatic progenitors express Pdx1

A group of special cells in the thickened DE epithelium along the dorsal and ventral surfaces of the posterior

mouse foregut at E9.0-9.5 expresses the gene named *Pdx1* (pancreas and duodenal homeobox 1, also known as *IPF1*, insulin promoter factor 1 in humans). Pdx1 is a transcription factor of the paralogous homeobox family and is essential for both the expansion of pancreas primordial populations<sup>[26]</sup> and the function of adult  $\beta$  cells<sup>[27,28]</sup>. Genetic lineage tracing experiments demonstrated that pancreatic Pdx1-expressing (Pdx1<sup>+</sup>) progenitors give rise to acinar, duct and endocrine tissues in the pancreas<sup>[29]</sup>. These progenitors are located at the tip of the branching pancreatic tree marked by Pdx1<sup>+</sup>Ptf1a<sup>+</sup> (pancreas transcription factor 1a) Cpa1<sup>+</sup> (carboxypeptidase 1)<sup>[30]</sup>. Replacement of most of the homeodomain of PDX-1 with the lacZ reporter, allows visualization of the PDX-1/ $\beta$ -galactosidase fusion allele, and it was found to be expressed in pancreatic, duodenal and antral stomach lineages<sup>[31]</sup>. The non-pancreas endoderm-derived expression of Pdx1 was established with the application of a different labeling strategy<sup>[32]</sup>. These studies suggest that PSC-derived Pdx1<sup>+</sup> cells may commit to any of these lineages. Thus, caution should be taken because all PSC-derived Pdx1<sup>+</sup> cells may not be the equivalent of the pancreatic Pdx1<sup>+</sup> progenitors.

In humans, numerous PDX1<sup>+</sup> progenitors can be detected easily in the developing pancreas between 8 and 21 wk of age<sup>[33,34]</sup>. These PDX1<sup>+</sup> progenitors frequently express SOX9 and are highly proliferative<sup>[35]</sup>, supporting the notion that PDX1<sup>+</sup> progenitors are committed from SOX9<sup>+</sup> multipotent progenitors. The number of PDX1<sup>+</sup> cells that also express insulin or somatostatin progressively increases during this period of development<sup>[33]</sup>. An unanswered fundamental question is the origin of the PDX1<sup>+</sup>

progenitors: Are they generated by self-renewal, or by commitment from their endodermal progenitors, or from both sources?

Following *in vivo* developmental pathways, PSCs can be directed to give rise to Pdx1<sup>+</sup> cells in the presence of the protein kinase C activator indolactam (ILV)<sup>[36]</sup>. These cells are able to proliferate 16-fold in the presence of pancreas-derived mesenchymal cells<sup>[37]</sup>. Independent confirmation of these results is essential to verify this capacity of the Pdx1<sup>+</sup> cells. It is also important to address whether all or only a minor fraction of PSC-derived Pdx1<sup>+</sup> cells commit along the endocrine pathway. To resolve these issues, identification of a specific marker that allows the purification of the Pdx1<sup>+</sup> pancreatic progenitor-like cells would be valuable.

### ***Ngn3-expressing islet progenitors***

At around E9.5 in mice, a small group of cells in the thickened posterior foregut DE epithelium begins to express the basic helix-loop-helix transcription factor neurogenin 3 (Ngn3, also known as neurog3)<sup>[29,38,39]</sup>. These Ngn3<sup>+</sup> cells are islet progenitors because they can give rise to all islet lineage cells. Whereas mouse *Ngn3* mRNA expression in the developing pancreas peaks around E15.5<sup>[40]</sup> (equivalent to week 9 in humans), human *NGN3* expression is low before 9 wk, from which time, its expression increases sharply and remains high until 17 wk<sup>[34]</sup>.

A number of observations support the importance of Ngn3 in islet development: Islet cells are not observed in *Ngn3* knockout mice<sup>[38]</sup>; gene lineage tracing demonstrates that Ngn3<sup>+</sup> progenitors give rise to all pancreatic endocrine cells<sup>[29]</sup>; in adult pancreas, purified Ngn3<sup>+</sup> cells activated by pancreatic duct ligation (PDL) can, after injection into a fetal pancreas *in vitro*, differentiate into all islet cell types<sup>[39]</sup>. In contrast, one group reported that although PDL allows activation of Ngn3 expression, the Ngn3<sup>+</sup> cells were not able to complete the entire  $\beta$ -cell developmental program<sup>[41]</sup> and a more recent study found that  $\beta$ -cell mass and insulin content were totally unchanged by PDL-induced injury<sup>[42]</sup>. The reason for these inconsistencies is unknown so future studies are required to resolve this matter.

Interestingly, insulin protein has been detected in islet progenitors in the developing human and mouse pancreas. In a dual fluorescence reporter mouse line, a few Ngn3<sup>+</sup> cells in the developing pancreas coexpress insulin<sup>[43]</sup>. In humans, some NGN3<sup>+</sup> cells were also detected to coexpress insulin in the fetal pancreas between 10 and 21 wk<sup>[33]</sup>. Recently, inhibitors of vesicular monoamine transporter-2 (reserpine and tetrabenzine, TBZ), were shown to mediate differentiation of PSC-derived Pdx1<sup>+</sup> cells into *Ngn3*-expressing cells<sup>[44]</sup>. Again, caution has to be taken regarding the use of genetic lineage tracing in PSC differentiation because successful *in vivo* lineage tracing studies rely on the temporospatial cues (see review<sup>[45]</sup>) and Ngn3-expressing cells are present in multiple tissues including the endoderm-derived intestine<sup>[46]</sup>. Despite lineage tracing demonstrating

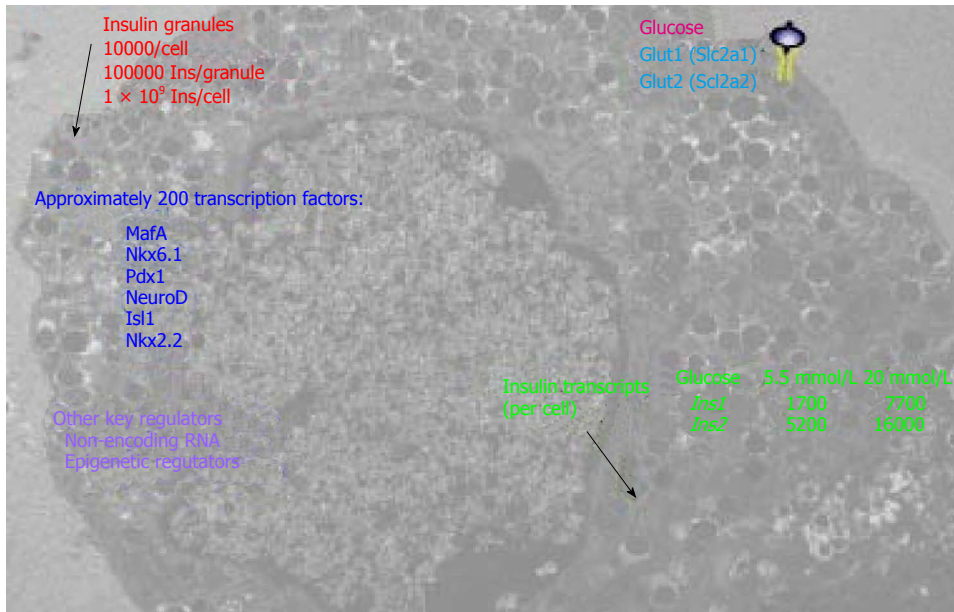
that Ngn3<sup>+</sup> cells will complete the differentiation process prenatally to all pancreatic endocrine cells including  $\beta$  cells<sup>[29]</sup>, these only become glucose-responsive postnatally.

## **INSULIN-SECRETING $\beta$ CELLS**

In adults, there are approximately 1000 endocrine islets in mice and  $1 \times 10^6$  in humans distributed throughout a healthy pancreas, representing up to 2% of the total mass of the organ<sup>[47]</sup>. Each islet varies in size from 100 to 500  $\mu$ m in diameter and is made up of 1000-3000 cells<sup>[48]</sup>. In rodents,  $\beta$  cells are the major component, accounting for up to 80% of the total number in the islets, with the remainder comprised of  $\alpha$  cells (approximately 15%) and the remaining endocrine  $\delta$ , PP and  $\epsilon$  cells (approximately 5%). In the human islet, the proportions of  $\delta$  and PP cells are similar, but  $\beta$  cells are less abundant (48%-59%) and the  $\alpha$ -cell population accounts a 33%-46%<sup>[49]</sup>. Interestingly, a substantial number of  $\epsilon$  cells are found in adult islets in humans, but not in other known species<sup>[50]</sup>.

Insulin orchestrates blood glucose utilization by peripheral metabolic tissues such as the liver, muscle and adipose tissue, while glucagon raises blood glucose concentrations by acting on the liver, brain, adipose tissue and heart<sup>[51]</sup>. Thus both hormones are critical in maintaining glucose homeostasis. A close paracrine regulatory loop is present between  $\alpha$  and  $\beta$  cells. For example,  $\beta$  cells secrete urocortin 3 to stimulate the release of somatostatin which in turn suppresses secreting glucagon from  $\alpha$  cells<sup>[52]</sup>;  $\alpha$  cells also generate ghrelin, which is normally believed to be produced by  $\epsilon$  cells, to inhibit insulin secretion but stimulate their own glucagon secretion<sup>[53]</sup>.

Clearly, the  $\beta$  cell is a highly effective and efficient factory specialized for the production of insulin. For example, on average a rodent  $\beta$  cell contains approximately 10000 insulin granules (Figure 3), corresponding to approximately 10%-20% of the total cell volume. Each granule stores approximately  $2 \times 10^5$  insulin molecules, thus a  $\beta$  cell could package  $2 \times 10^9$  insulin molecules<sup>[48]</sup>. At least 17 key transcription factors (including FOXA2, FOXO1, HNF1A, INSM1, ISL1, MAFA, MNX1, MYT1, NEUROD1, NKX2.2, NKX6.1, PAX6, PDX1, RFX6 TCF7L2 and RFX3) are required to maintain  $\beta$ -cell function<sup>[54]</sup>; some of these are shown in Figure 3. The basic-leucine zipper transcription factor MAFA (musculoaponeurotic fibrosarcoma oncogene family protein A), for example, is an important *INS* transactivator<sup>[55]</sup>. PDX1 is well known to activate and maintain *INS* and *GLUT2* (glucose transporter 2) expression in  $\beta$  cells<sup>[56,57]</sup>. A gene network controlled by NKX6.1 is essential for maintaining the functional and molecular traits of mature  $\beta$  cells<sup>[58]</sup>. Pancreatic  $\beta$  cells require NEUROD1 (neuronal differentiation 1) to achieve and maintain a functional state<sup>[59]</sup> by DNA methylation-mediated repression of the lineage determination gene *aristaless-related homeobox*<sup>[60]</sup>. In addition to key transcription factors, the fractalkine (also known as CXCL1 or neurotoxin)/CXCL1R (also known as GPR13) system



**Figure 3** An integral view of insulin-secreting  $\beta$  cells. The highly specialized cells have a powerful function that is regulated by multiple layers of signaling.

also regulates  $\beta$ -cell function and insulin secretion<sup>[61]</sup>. Furthermore,  $\beta$  cells develop a highly sophisticated electrophysiology<sup>[62]</sup> and glucose sensing system for blood glucose concentrations for fine-tuned secretion of insulin granules to maintain normal glucose homeostasis, which is critical for normal physiology of many pivotal organs.

Recently, the application of high throughput RNA and DNA sequencing technologies has given us a more integral view of insulin-secreting  $\beta$  cells. Deep RNA sequencing of purified human  $\beta$  cells demonstrated *INS* is the most abundantly transcribed gene, representing approximately 38% of the  $\beta$ -cell transcriptome<sup>[63]</sup>, within which also contains transcripts from over 9900 other genes<sup>[64]</sup>. Massively parallel signature sequencing demonstrated that there are over 200  $\beta$ -cell specific transcription factor genes<sup>[65]</sup> that regulate this fine-tuned function. Uniquely, the human *INS* gene is marked by high levels of histone acetylation and H3K4 demethylation at around approximately 80 kb from the transcription start site. These modifications in many other human genes are concentrated around only 1 kb of the start site<sup>[66]</sup>. Consistently, high-throughput sequencing of formaldehyde-assisted isolation of regulatory elements (FAIRE-seq) identified approximately 3300 human islet-selective open chromatin sites<sup>[67]</sup>. Polyadenylated mRNA sequencing reveals that over 1000 long intergenic noncoding RNA species are transcribed in mouse and human  $\beta$  cells<sup>[68,69]</sup>. A review of transcriptomes and other omics of  $\beta$  cells can be found elsewhere<sup>[70]</sup>.

## CONFUSION IN THE CONCEPT OF $\beta$ CELLS

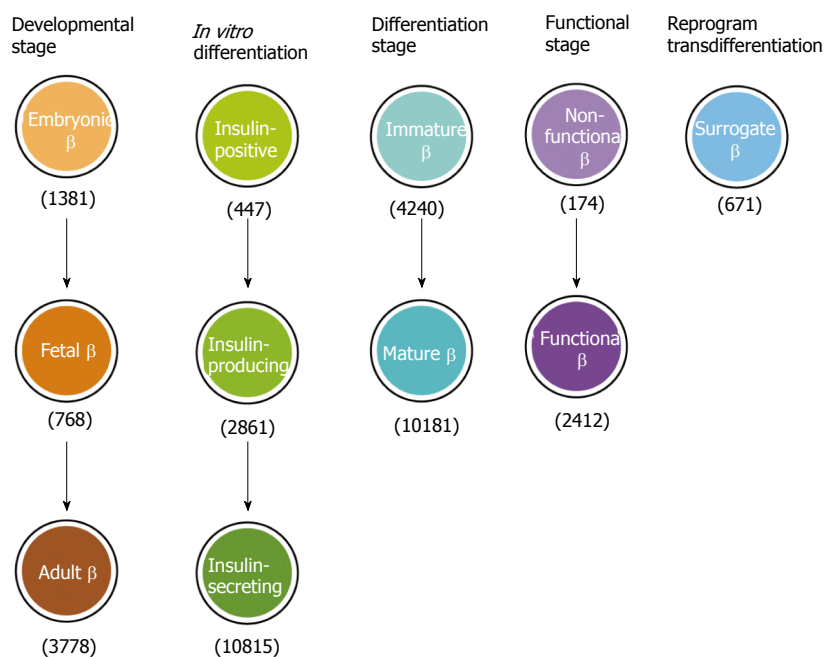
Reductionist approaches applied over the last two decades have uncovered a complex transcription regulatory net-

work for islet lineage development<sup>[71,72]</sup>. Despite the fact that intense international efforts have concentrated on differentiation of PSCs for replacing/restoring the lost  $\beta$  cell function, application of this knowledge for translational research to produce functional  $\beta$  cells *in vitro* has not been straightforward. This is because knowledge generated from *in vivo* studies in rodent models is not necessarily applicable to *in vitro* studies, in particular for human cells. Over this period, at least 11 nomenclatures and definitions have been given to insulin-producing cells (Figure 4) that were generally believed to be the equivalent of *in vivo*  $\beta$  cells.

As the pancreatic islet population and neural cells share a large number of markers and perhaps mechanisms of differentiation<sup>[73]</sup>, mouse ESCs were early reported to give rise to insulin-positive cells in culture conditions that were used for neural cell differentiation<sup>[74]</sup>. Although the differentiated cells were stained positive for insulin, it was subsequently shown this was due to the uptake of insulin from the culture medium rather than the activation of robust insulin transcription<sup>[75]</sup>. Additionally, there were several reports of generating pancreatic endocrine cells or functional  $\beta$  cells from PSCs<sup>[76-78]</sup>. Later these cells were however demonstrated to be similar to fetal  $\beta$  cells<sup>[79]</sup> and to lack the transcriptomic and epigenetic profiles of adult islet cells<sup>[80]</sup>.

Nevertheless in such a short timeframe, PSCs have been convincingly differentiated following their normal *in vivo* developmental mechanisms into cells of approximately at the pancreatic progenitor and/or islet progenitor stages<sup>[21,36,37,80-84]</sup>. In contrast, due to a lack of knowledge of the late stage pancreatic endocrine lineage<sup>[85,86]</sup>, empirical protocols have been used for their further differentiation. Inevitably the PSC-derived endocrine populations may only contain a small fraction of genuine insulin-secreting cells or are immature, as reversal of diabetes in mice requires





**Figure 4 Conceptual confusions in insulin-secreting  $\beta$  cells.** Insulin-secreting cells have been given a variety of nomenclature depending on the developmental stages, *in vitro* differentiation, functional states or reprogram/transdifferentiation. The number of used nomenclature in parenthesis was from a Pubmed search in May 2015.

five million SC- $\beta$  cells<sup>[87]</sup> or further maturation *in vivo*<sup>[88]</sup>. Readers are referred to recent fine reviews regarding the current state and problems on PSC differentiation towards pancreatic endocrine cells<sup>[20,86,89-91]</sup>. Perhaps the problems and confusions on the concept of insulin-secreting  $\beta$  cells seem to have produced negative impacts in the academic community while generating unhelpful excitement and expectations on the reality of future diabetes regenerative medicine to the general public. Furthermore, the confusion and controversy has hampered the progress of not only the field of islet developmental biology but also the establishment of a regenerative therapy to diabetes *per se*. The following section exemplifies several potential, but not exclusive, causes of the confusion and controversy.

#### The presence of extrapancreas insulin-producing cells

Making the issues more complicated, multiple sites in the body can produce insulin. The thymus, another foregut-derived organ (Figure 2), for example, normally produces insulin, in order to induce self-tolerance and protection of the body from the autoimmune destruction of pancreatic insulin-secreting  $\beta$  cells<sup>[92]</sup> as thymus-specific deletion of insulin results in both autoimmune destruction of these cells and diabetes<sup>[93]</sup>. Certain areas of the brain also express the insulin gene and produce insulin protein<sup>[94]</sup> and these share several transcription factors of the islet lineage<sup>[73]</sup>. In different diabetic models, including streptozotocin-treated mice and rats, ob/ob mice, and mice fed high-fat diets, insulin mRNA and protein expression have been detected in the liver, adipose tissue, spleen, bone marrow as well as thymus<sup>[95]</sup>. An interesting question is whether these extrapancreatic insulin-producing cells are able to give rise *in vitro* to functional insulin-secreting cells. Otherwise, such extrapancreatic insulin-producing cells are simply non-functional cells.

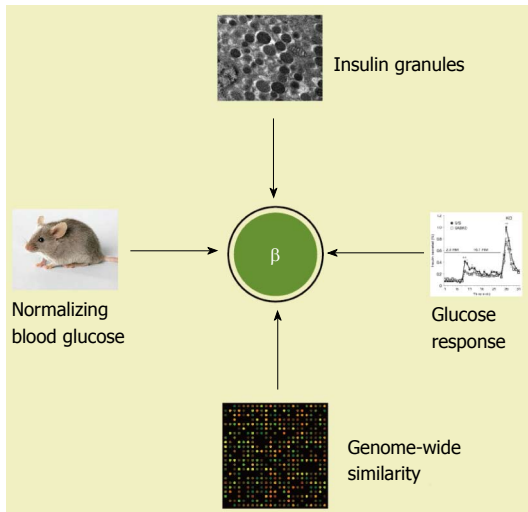
Taken together, these data suggest that PSC-derived insulin-producing cells might consist of physiologically irrelevant insulin-producing cells.

#### Multiple fate commitments may accumulate non-functional insulin-producing cells

PSCs theoretically have the capacity to give rise to all of the functionally-defined 210 cell types in the body, so to induce them to becoming desirable  $\beta$  cells requires forcing them to make multiple fate commitments under the guidance of exogenous differentiation factors (Figure 2). Treatment with these factors of course is not always 100% effective, resulting in some cells differentiating along unwanted pathways, even giving rise to non-functional insulin-producing cells especially in suboptimal or abnormal differentiation conditions. Currently, there is no documentation on whether any PSC-derived insulin-producing cells in the differentiated product are similar to those of extrapancreas-derived ones.

#### Empirical protocol may generate non-functional insulin-producing cells

The lack of knowledge of differentiation of late stage islet lineages<sup>[85,86]</sup> led researchers to develop cocktail protocols containing factors that have not been well-characterized. Development of such protocols depends heavily on the experience of researchers and poorly characterized combinations of factors may promote generation of non-functional insulin-producing cells. A better understanding of the  $\beta$ -cell differentiation pathway and its underlying mechanisms would therefore allow the establishment of a standardized directed differentiation protocol and stage-specific differentiation strategies, so that generation of non-functional insulin-producing cells could be minimized or avoided.



**Figure 5** A post-genomic concept of insulin-secreting  $\beta$  cells. Whereas four criteria are proposed, a highly similar transcriptomic profile to adult  $\beta$  cells is essential to establish pluripotent stem cells-derived  $\beta$  cells.

## $\beta$ CELLS REQUIRE A POST-GENOMIC CONCEPT

A major obstacle/challenge in defining PSC-derived insulin-secreting  $\beta$  cells is that the temporospatial cues that help identify these *in vivo* are absent in differentiation *in vitro*. As insulin is only a member of an insulin-related family<sup>[96,97]</sup>, it is critical to absolutely exclude whether any of the insulin antibodies (especially polyclonals) that have been used to characterize “insulin-producing cells” do not cross-react with other members of this family or even with other polypeptides. This is because “antibodies often recognize extra proteins in addition to the ones they are told to detect” and their reproducibility needs to be dramatically improved<sup>[98,99]</sup>.

We propose at least four essential criteria for insulin-secreting  $\beta$  cells for further discussions and considerations. Compared to adult  $\beta$  cells, the *in vitro* PSC-derived cells must have: (1) An equivalent number of insulin granules under electron microscopy; (2) a similar dynamic glucose stimulated insulin secretion; (3) a highly similar transcriptomic profile (not a similarity in a selected gene profile of transcriptomic datasets), and (4) the capability to normalize hyperglycemia within a few weeks after transplantation as an equivalent number of functional  $\beta$  cells do (Figure 5).

Definition of functional insulin-secreting  $\beta$  cells at the transcriptomic level is an essential requirement. Alternatively, single-cell transcriptomic and epigenomic analyses of PSC-derived insulin-producing cells could help establish this concept.

## CONCLUSION

Currently the sophisticated insulin pump also known as the “Closed Loop Therapy” or “Artificial Pancreas” can deliver insulin in a precise manner, resulting in a significant

improvement in the blood glucose control and the quality of life for people with diabetes<sup>[100,101]</sup>. Perhaps we should exercise extra caution for stem cell therapies to diabetes, due to the concern of tumorigenesis<sup>[102]</sup>, off-target differentiation<sup>[89]</sup>, biosafety and reliability having not yet been convincingly addressed. The application of genomic, epigenomic, transcriptomic, and/or proteomic approaches to characterize differentiated products will not only verify their safety profile and differentiated state but also shed light on their transcription regulation and molecular mechanisms. The pharmaceutical and biotechnological sectors should work together with the academic community to strengthen fundamental research, identify ways to purify/enrich PSC-derived progenitors at specific stages and develop directed differentiation protocols for the development of the stage-specific progenitors towards genuine insulin-secreting  $\beta$  cells. The progenitors at different stages and differentiated insulin-secreting cells would also be useful for fundamental research and drug screening. Thus, the ability to generate the highly specialized functional  $\beta$  cells *in vitro* will not only generate new knowledge of pancreatic endocrine lineages, but also provide a critical cell source for a diabetes regenerative therapy, a potentially robust and better medicine. In doing so, safe, stable, reliable and functional cellular products will ultimately be available to people with T1D and those with some forms of T2D.

## REFERENCES

- 1 **Halban PA**, Polonsky KS, Bowden DW, Hawkins MA, Ling C, Mather KJ, Powers AC, Rhodes CJ, Sussel L, Weir GC.  $\beta$ -cell failure in type 2 diabetes: postulated mechanisms and prospects for prevention and treatment. *J Clin Endocrinol Metab* 2014; **99**: 1983-1992 [PMID: 24712577 DOI: 10.1210/jc.2014-1425]
- 2 **Jiang FX**, Morahan G. Pancreatic stem cells remain unresolved. *Stem Cells Dev* 2014; **23**: 2803-2812 [PMID: 25132582 DOI: 10.1089/scd.2014.0214]
- 3 **Thomson JA**, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. *Science* 1998; **282**: 1145-1147 [PMID: 9804556 DOI: 10.1126/science.282.5391.1145]
- 4 **Shapiro AM**, Lakey JR, Ryan EA, Korbitt GS, Toth E, Warnock GL, Kneteman NM, Rajotte RV. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 2000; **343**: 230-238 [PMID: 10911004 DOI: 10.1056/NEJM200007273430401]
- 5 **Falkmer S**, Patent GT. Comparative and embryological aspects of the pancreatic islets. *Handbook of Physiology*. In: John F, editor. Washington: American Physiological Society, 1972: 1-23
- 6 **Banting FG**, Best CH, Collip JB, Campbell WR, Fletcher AA. Pancreatic Extracts in the Treatment of Diabetes Mellitus. *Can Med Assoc J* 1922; **12**: 141-146 [PMID: 20314060]
- 7 **Lacy PE**, Davies J. Preliminary studies on the demonstration of insulin in the islets by the fluorescent antibody technic. *Diabetes* 1957; **6**: 354-357 [PMID: 13447765 DOI: 10.2337/diab.6.4.354]
- 8 **Brown H**, Sanger F, Kitai R. The structure of pig and sheep insulins. *Biochem J* 1955; **60**: 556-565 [PMID: 13249948 DOI: 10.1042/bj0600556]
- 9 **Ullrich A**, Shine J, Chirgwin J, Pictet R, Tischer E, Rutter WJ, Goodman HM. Rat insulin genes: construction of plasmids containing the coding sequences. *Science* 1977; **196**: 1313-1319 [PMID: 325648 DOI: 10.1126/science.325648]
- 10 **Pictet RL**, Clark WR, Williams RH, Rutter WJ. An ultrastructural

- analysis of the developing embryonic pancreas. *Dev Biol* 1972; **29**: 436-467 [PMID: 4570759 DOI: 10.1016/0012-1606(72)90083-8]
- 11 **Piper K**, Brickwood S, Turnpenny LW, Cameron IT, Ball SG, Wilson DI, Hanley NA. Beta cell differentiation during early human pancreas development. *J Endocrinol* 2004; **181**: 11-23 [PMID: 15072563 DOI: 10.1677/joe.0.1810011]
- 12 **Herrera PL**. Adult insulin- and glucagon-producing cells differentiate from two independent cell lineages. *Development* 2000; **127**: 2317-2322 [PMID: 10804174]
- 13 **Herrera PL**, Huarte J, Sanvito F, Meda P, Orci L, Vassalli JD. Embryogenesis of the murine endocrine pancreas; early expression of pancreatic polypeptide gene. *Development* 1991; **113**: 1257-1265 [PMID: 1811941]
- 14 **Richardson MK**, Hanken J, Gooneratne ML, Pieau C, Raynaud A, Selwood L, Wright GM. There is no highly conserved embryonic stage in the vertebrates: implications for current theories of evolution and development. *Anat Embryol (Berl)* 1997; **196**: 91-106 [PMID: 9278154 DOI: 10.1007/s004290050082]
- 15 **Fougerousse F**, Bullen P, Herasse M, Lindsay S, Richard I, Wilson D, Suel L, Durand M, Robson S, Abitbol M, Beckmann JS, Strachan T. Human-mouse differences in the embryonic expression patterns of developmental control genes and disease genes. *Hum Mol Genet* 2000; **9**: 165-173 [PMID: 10607827 DOI: 10.1093/hmg/9.2.165]
- 16 **Lukinius A**, Ericsson JL, Grimelius L, Korsgren O. Ultrastructural studies of the ontogeny of fetal human and porcine endocrine pancreas, with special reference to colocalization of the four major islet hormones. *Dev Biol* 1992; **153**: 376-385 [PMID: 1356860 DOI: 10.1016/0012-1606(92)90122-W]
- 17 **Polak M**, Bouchareb-Banaei L, Scharfmann R, Czemichow P. Early pattern of differentiation in the human pancreas. *Diabetes* 2000; **49**: 225-232 [PMID: 10868939 DOI: 10.2337/diabetes.49.2.225]
- 18 **Pan FC**, Brissova M. Pancreas development in humans. *Curr Opin Endocrinol Diabetes Obes* 2014; **21**: 77-82 [PMID: 24569548 DOI: 10.1097/MED.0000000000000047]
- 19 **De Krijger RR**, Aanstoot HJ, Kranenburg G, Reinhard M, Visser WJ, Bruining GJ. The midgestational human fetal pancreas contains cells coexpressing islet hormones. *Dev Biol* 1992; **153**: 368-375 [PMID: 1356859 DOI: 10.1016/0012-1606(92)90121-V]
- 20 **Nair G**, Hebrok M. Islet formation in mice and men: lessons for the generation of functional insulin-producing  $\beta$ -cells from human pluripotent stem cells. *Curr Opin Genet Dev* 2015; **32**: 171-180 [PMID: 25909383 DOI: 10.1016/j.gde.2015.03.004]
- 21 **Cheng X**, Ying L, Lu L, Galvão AM, Mills JA, Lin HC, Kotton DN, Shen SS, Nostro MC, Choi JK, Weiss MJ, French DL, Gadue P. Self-renewing endodermal progenitor lines generated from human pluripotent stem cells. *Cell Stem Cell* 2012; **10**: 371-384 [PMID: 22482503 DOI: 10.1016/j.stem.2012.02.024]
- 22 **Li K**, Zhu S, Russ HA, Xu S, Xu T, Zhang Y, Ma T, Hebrok M, Ding S. Small molecules facilitate the reprogramming of mouse fibroblasts into pancreatic lineages. *Cell Stem Cell* 2014; **14**: 228-236 [PMID: 24506886 DOI: 10.1016/j.stem.2014.01.006]
- 23 **Sarkar A**, Hochedlinger K. The sox family of transcription factors: versatile regulators of stem and progenitor cell fate. *Cell Stem Cell* 2013; **12**: 15-30 [PMID: 23290134 DOI: 10.1016/j.stem.2012.12.007]
- 24 **Lynn FC**, Smith SB, Wilson ME, Yang KY, Nekrep N, German MS. Sox9 coordinates a transcriptional network in pancreatic progenitor cells. *Proc Natl Acad Sci USA* 2007; **104**: 10500-10505 [PMID: 17563382]
- 25 **Furuyama K**, Kawaguchi Y, Akiyama H, Horiguchi M, Kodama S, Kuhara T, Hosokawa S, Elbahrawy A, Soeda T, Koizumi M, Masui T, Kawaguchi M, Takaori K, Doi R, Nishi E, Kakinoki R, Deng JM, Behringer RR, Nakamura T, Uemoto S. Continuous cell supply from a Sox9-expressing progenitor zone in adult liver, exocrine pancreas and intestine. *Nat Genet* 2011; **43**: 34-41 [PMID: 21113154 DOI: 10.1038/ng.722]
- 26 **Jonsson J**, Carlsson L, Edlund T, Edlund H. Insulin-promoter-factor 1 is required for pancreas development in mice. *Nature* 1994; **371**: 606-609 [PMID: 7935793 DOI: 10.1038/371606a0]
- 27 **Ohneda K**, Mirmira RG, Wang J, Johnson JD, German MS. The homeodomain of PDX-1 mediates multiple protein-protein interactions in the formation of a transcriptional activation complex on the insulin promoter. *Mol Cell Biol* 2000; **20**: 900-911 [PMID: 10629047 DOI: 10.1128/MCB.20.3.900-911.2000]
- 28 **Gao T**, McKenna B, Li C, Reichert M, Nguyen J, Singh T, Yang C, Pannikar A, Doliba N, Zhang T, Stoffers DA, Edlund H, Matschinsky F, Stein R, Stanger BZ. Pdx1 maintains  $\beta$  cell identity and function by repressing an  $\alpha$  cell program. *Cell Metab* 2014; **19**: 259-271 [PMID: 24506867 DOI: 10.1016/j.cmet.2013.12.002]
- 29 **Gu G**, Dubauskaite J, Melton DA. Direct evidence for the pancreatic lineage: NGN3+ cells are islet progenitors and are distinct from duct progenitors. *Development* 2002; **129**: 2447-2457 [PMID: 11973276]
- 30 **Zhou Q**, Law AC, Rajagopal J, Anderson WJ, Gray PA, Melton DA. A multipotent progenitor domain guides pancreatic organogenesis. *Dev Cell* 2007; **13**: 103-114 [PMID: 17609113 DOI: 10.1016/j.devcel.2007.06.001]
- 31 **Offield MF**, Jetton TL, Labosky PA, Ray M, Stein RW, Magnuson MA, Hogan BL, Wright CV. PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. *Development* 1996; **122**: 983-995 [PMID: 8631275]
- 32 **Holland AM**, Góñez LJ, Naselli G, Macdonald RJ, Harrison LC. Conditional expression demonstrates the role of the homeodomain transcription factor Pdx1 in maintenance and regeneration of beta-cells in the adult pancreas. *Diabetes* 2005; **54**: 2586-2595 [PMID: 16123346 DOI: 10.2337/diabetes.54.9.2586]
- 33 **Lyttle BM**, Li J, Krishnamurthy M, Fellows F, Wheeler MB, Goodyer CG, Wang R. Transcription factor expression in the developing human fetal endocrine pancreas. *Diabetologia* 2008; **51**: 1169-1180 [PMID: 18491072 DOI: 10.1007/s00125-008-1006-z]
- 34 **Jeon J**, Correa-Medina M, Ricordi C, Edlund H, Diez JA. Endocrine cell clustering during human pancreas development. *J Histochem Cytochem* 2009; **57**: 811-824 [PMID: 19365093 DOI: 10.1369/jhc.2009.953307]
- 35 **McDonald E**, Li J, Krishnamurthy M, Fellows GF, Goodyer CG, Wang R. SOX9 regulates endocrine cell differentiation during human fetal pancreas development. *Int J Biochem Cell Biol* 2012; **44**: 72-83 [PMID: 21983268 DOI: 10.1016/j.biocel.2011.09.008]
- 36 **Chen S**, Borowiak M, Fox JL, Maehr R, Osafune K, Davidow L, Lam K, Peng LF, Schreiber SL, Rubin LL, Melton D. A small molecule that directs differentiation of human ESCs into the pancreatic lineage. *Nat Chem Biol* 2009; **5**: 258-265 [PMID: 19287398 DOI: 10.1038/nchembio.154]
- 37 **Sneddon JB**, Borowiak M, Melton DA. Self-renewal of embryonic-stem-cell-derived progenitors by organ-matched mesenchyme. *Nature* 2012; **491**: 765-768 [PMID: 23041930 DOI: 10.1038/nature11463]
- 38 **Gradwohl G**, Dierich A, LeMeur M, Guillemot F. neurogenin3 is required for the development of the four endocrine cell lineages of the pancreas. *Proc Natl Acad Sci USA* 2000; **97**: 1607-1611 [PMID: 10677506]
- 39 **Xu X**, D'Hoker J, Stangé G, Bonnè S, De Leu N, Xiao X, Van de Casteele M, Mellitzer G, Ling Z, Pipeleers D, Bouwens L, Scharfmann R, Gradwohl G, Heimberg H. Beta cells can be generated from endogenous progenitors in injured adult mouse pancreas. *Cell* 2008; **132**: 197-207 [PMID: 18243096 DOI: 10.1016/j.cell.2007.12.015]
- 40 **Schwitzgebel VM**, Scheel DW, Connors JR, Kalamaras J, Lee JE, Anderson DJ, Sussel L, Johnson JD, German MS. Expression of neurogenin3 reveals an islet cell precursor population in the pancreas. *Development* 2000; **127**: 3533-3542 [PMID: 10903178]
- 41 **Kopp JL**, Dubois CL, Schaffer AE, Hao E, Shih HP, Seymour PA, Ma J, Sander M. Sox9+ ductal cells are multipotent progenitors throughout development but do not produce new endocrine cells in the normal or injured adult pancreas. *Development* 2011; **138**: 653-665 [PMID: 21266405 DOI: 10.1242/dev.056499]
- 42 **Rankin MM**, Wilbur CJ, Rak K, Shields EJ, Granger A, Kushner JA.  $\beta$ -Cells are not generated in pancreatic duct ligation-induced injury in adult mice. *Diabetes* 2013; **62**: 1634-1645 [PMID: 23444444 DOI: 10.2337/db12-1000]



- 23349489 DOI: 10.2337/db12-0848]
- 43 **Hara M**, Dizon RF, Glick BS, Lee CS, Kaestner KH, Piston DW, Bindokas VP. Imaging pancreatic beta-cells in the intact pancreas. *Am J Physiol Endocrinol Metab* 2006; **290**: E1041-E1047 [PMID: 16368785 DOI: 10.1152/ajpendo.00365.2005]
  - 44 **Sakano D**, Shiraki N, Kikawa K, Yamazoe T, Kataoka M, Umeda K, Araki K, Mao D, Matsumoto S, Nakagata N, Andersson O, Stainier D, Endo F, Kume K, Uesugi M, Kume S. VMAT2 identified as a regulator of late-stage  $\beta$ -cell differentiation. *Nat Chem Biol* 2014; **10**: 141-148 [PMID: 24316738 DOI: 10.1038/nchembio.1410]
  - 45 **Jiang FX**, Morahan G. Directed differentiation of late stage islet lineages remains a knowledge gap in pancreatic endocrine development. *JJ Bone Stem Res* 2015; **1**: 002
  - 46 **Jenny M**, Uhl C, Roche C, Duluc I, Guillermin V, Guillemot F, Jensen J, Kedinger M, Gradwohl G. Neurogenin3 is differentially required for endocrine cell fate specification in the intestinal and gastric epithelium. *EMBO J* 2002; **21**: 6338-6347 [PMID: 12456641 DOI: 10.1093/emboj/cdf649]
  - 47 **Quesada I**, Tudurí E, Ripoll C, Nadal A. Physiology of the pancreatic alpha-cell and glucagon secretion: role in glucose homeostasis and diabetes. *J Endocrinol* 2008; **199**: 5-19 [PMID: 18669612 DOI: 10.1677/JOE-08-0290]
  - 48 **Suckale J**, Solimena M. The insulin secretory granule as a signaling hub. *Trends Endocrinol Metab* 2010; **21**: 599-609 [PMID: 20609596 DOI: 10.1016/j.tem.2010.06.003]
  - 49 **Cabrera O**, Berman DM, Kenyon NS, Ricordi C, Berggren PO, Caicedo A. The unique cytoarchitecture of human pancreatic islets has implications for islet cell function. *Proc Natl Acad Sci USA* 2006; **103**: 2334-2339 [PMID: 16461897 DOI: 10.1073/pnas.0510790103]
  - 50 **Wierup N**, Sundler F, Heller RS. The islet ghrelin cell. *J Mol Endocrinol* 2014; **52**: R35-R49 [PMID: 24049065 DOI: 10.1530/JME-13-0122]
  - 51 **Campbell JE**, Drucker DJ. Islet  $\alpha$  cells and glucagon--critical regulators of energy homeostasis. *Nat Rev Endocrinol* 2015; **11**: 329-338 [PMID: 25850661 DOI: 10.1038/nrendo.2015.51]
  - 52 **van der Meulen T**, Donaldson CJ, Cáceres E, Hunter AE, Cowing-Zitron C, Pound LD, Adams MW, Zembrzycki A, Grove KL, Huising MO. Urocortin3 mediates somatostatin-dependent negative feedback control of insulin secretion. *Nat Med* 2015; **21**: 769-776 [PMID: 26076035 DOI: 10.1038/nm.3872]
  - 53 **Chuang JC**, Sakata I, Kohno D, Perello M, Osborne-Lawrence S, Repa JJ, Zigman JM. Ghrelin directly stimulates glucagon secretion from pancreatic alpha-cells. *Mol Endocrinol* 2011; **25**: 1600-1611 [PMID: 21719535 DOI: 10.1210/me.2011-1001]
  - 54 **Pasquali L**, Gaulton KJ, Rodríguez-Seguí SA, Mularoni L, Miguel-Escalada I, Akerman I, Tena JJ, Morán I, Gómez-Marín C, van de Bunt M, Ponsa-Cobas J, Castro N, Nammo T, Cebola I, García-Hurtado J, Maestro MA, Pattou F, Piemonti L, Berney T, Gloyn AL, Ravassard P, Gómez-Skarmeta JL, Müller F, McCarthy MI, Ferrer J. Pancreatic islet enhancer clusters enriched in type 2 diabetes risk-associated variants. *Nat Genet* 2014; **46**: 136-143 [PMID: 24413736 DOI: 10.1038/ng.2870]
  - 55 **Sharma A**, Fusco-DeMane D, Henderson E, Efrat S, Stein R. The role of the insulin control element and RIPE3b1 activators in glucose-stimulated transcription of the insulin gene. *Mol Endocrinol* 1995; **9**: 1468-1476 [PMID: 8584024 DOI: 10.1210/mend.9.11.8584024]
  - 56 **Petersen HV**, Serup P, Leonard J, Michelsen BK, Madsen OD. Transcriptional regulation of the human insulin gene is dependent on the homeodomain protein STF1/IPF1 acting through the CT boxes. *Proc Natl Acad Sci USA* 1994; **91**: 10465-10469 [PMID: 7937976 DOI: 10.1073/pnas.91.22.10465]
  - 57 **Waeber G**, Thompson N, Nicod P, Bonny C. Transcriptional activation of the GLUT2 gene by the IPF-1/STF-1/IDX-1 homeobox factor. *Mol Endocrinol* 1996; **10**: 1327-1334 [PMID: 8923459 DOI: 10.1210/mend.10.11.8923459]
  - 58 **Taylor BL**, Liu FF, Sander M. Nkx6.1 is essential for maintaining the functional state of pancreatic beta cells. *Cell Rep* 2013; **4**: 1262-1275 [PMID: 24035389 DOI: 10.1016/j.celrep.2013.08.010]
  - 59 **Gu C**, Stein GH, Pan N, Goebbels S, Hörnberg H, Nave KA, Herrera P, White P, Kaestner KH, Sussel L, Lee JE. Pancreatic beta cells require NeuroD to achieve and maintain functional maturity. *Cell Metab* 2010; **11**: 298-310 [PMID: 20374962 DOI: 10.1016/j.cmet.2010.03.006]
  - 60 **Dhawan S**, Georgia S, Tschen SI, Fan G, Bhushan A. Pancreatic  $\beta$  cell identity is maintained by DNA methylation-mediated repression of Arx. *Dev Cell* 2011; **20**: 419-429 [PMID: 21497756 DOI: 10.1016/j.devcel.2011.03.012]
  - 61 **Lee YS**, Morinaga H, Kim JJ, Lagakos W, Taylor S, Keshwani M, Perkins G, Dong H, Kayali AG, Sweet IR, Olefsky J. The fractalkine/CX3CR1 system regulates  $\beta$  cell function and insulin secretion. *Cell* 2013; **153**: 413-425 [PMID: 23582329 DOI: 10.1016/j.cell.2013.03.001]
  - 62 **Rorsman P**, Braun M. Regulation of insulin secretion in human pancreatic islets. *Annu Rev Physiol* 2013; **75**: 155-179 [PMID: 22974438 DOI: 10.1146/annurev-physiol-030212-183754]
  - 63 **Nica AC**, Ongen H, Irminger JC, Bosco D, Berney T, Antonarakis SE, Halban PA, Dermitzakis ET. Cell-type, allelic, and genetic signatures in the human pancreatic beta cell transcriptome. *Genome Res* 2013; **23**: 1554-1562 [PMID: 23716500 DOI: 10.1101/gr.150706.112]
  - 64 **Benner C**, van der Meulen T, Cáceres E, Tigyi K, Donaldson CJ, Huising MO. The transcriptional landscape of mouse beta cells compared to human beta cells reveals notable species differences in long non-coding RNA and protein-coding gene expression. *BMC Genomics* 2014; **15**: 620 [PMID: 25051960 DOI: 10.1186/1471-2164-15-620]
  - 65 **Kutlu B**, Burdick D, Baxter D, Rasschaert J, Flamez D, Eizirik DL, Welsh N, Goodman N, Hood L. Detailed transcriptome atlas of the pancreatic beta cell. *BMC Med Genomics* 2009; **2**: 3 [PMID: 19146692 DOI: 10.1186/1755-8794-2-3]
  - 66 **Mutskov V**, Felsenfeld G. The human insulin gene is part of a large open chromatin domain specific for human islets. *Proc Natl Acad Sci USA* 2009; **106**: 17419-17424 [PMID: 19805079 DOI: 10.1073/pnas.0909288106]
  - 67 **Gaulton KJ**, Nammo T, Pasquali L, Simon JM, Giresi PG, Fogarty MP, Panhuis TM, Mieczkowski P, Secchi A, Bosco D, Berney T, Montanya E, Mohlke KL, Lieb JD, Ferrer J. A map of open chromatin in human pancreatic islets. *Nat Genet* 2010; **42**: 255-259 [PMID: 20118932 DOI: 10.1038/ng.530]
  - 68 **Ku GM**, Kim H, Vaughn IW, Hangauer MJ, Myung Oh C, German MS, McManus MT. Research resource: RNA-Seq reveals unique features of the pancreatic  $\beta$ -cell transcriptome. *Mol Endocrinol* 2012; **26**: 1783-1792 [PMID: 22915829 DOI: 10.1210/me.2012-1176]
  - 69 **Morán I**, Akerman I, van de Bunt M, Xie R, Benazra M, Nammo T, Arnes L, Nakić N, García-Hurtado J, Rodríguez-Seguí S, Pasquali L, Sauty-Colace C, Beucher A, Scharfmann R, van Arensbergen J, Johnson PR, Berry A, Lee C, Harkins T, Gmyr V, Pattou F, Kerr-Conte J, Piemonti L, Berney T, Hanley N, Gloyn AL, Sussel L, Langman L, Brayman KL, Sander M, McCarthy MI, Ravassard P, Ferrer J. Human  $\beta$  cell transcriptome analysis uncovers lncRNAs that are tissue-specific, dynamically regulated, and abnormally expressed in type 2 diabetes. *Cell Metab* 2012; **16**: 435-448 [PMID: 23040067 DOI: 10.1016/j.cmet.2012.08.010]
  - 70 **Blodgett DM**, Cura AJ, Harlan DM. The pancreatic  $\beta$ -cell transcriptome and integrated-omics. *Curr Opin Endocrinol Diabetes Obes* 2014; **21**: 83-88 [PMID: 24526012 DOI: 10.1097/MED.0000000000000051]
  - 71 **Seymour PA**, Sander M. Historical perspective: beginnings of the beta-cell: current perspectives in beta-cell development. *Diabetes* 2011; **60**: 364-376 [PMID: 21270248 DOI: 10.2337/db10-1068]
  - 72 **Pan FC**, Wright C. Pancreas organogenesis: from bud to plexus to gland. *Dev Dyn* 2011; **240**: 530-565 [PMID: 21337462 DOI: 10.1002/dvdy.22584]
  - 73 **Arntfield ME**, van der Kooy D.  $\beta$ -Cell evolution: How the pancreas borrowed from the brain: The shared toolbox of genes expressed by neural and pancreatic endocrine cells may reflect their



- evolutionary relationship. *Bioessays* 2011; **33**: 582-587 [PMID: 21681773 DOI: 10.1002/bies.201100015]
- 74 **Lumelsky N**, Blondel O, Laeng P, Velasco I, Ravin R, McKay R. Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. *Science* 2001; **292**: 1389-1394 [PMID: 11326082 DOI: 10.1126/science.1058866]
- 75 **Rajagopal J**, Anderson WJ, Kume S, Martinez OI, Melton DA. Insulin staining of ES cell progeny from insulin uptake. *Science* 2003; **299**: 363 [PMID: 12532008]
- 76 **D'Amour KA**, Bang AG, Eliazar S, Kelly OG, Agulnick AD, Smart NG, Moorman MA, Kroon E, Carpenter MK, Baetge EE. Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. *Nat Biotechnol* 2006; **24**: 1392-1401 [PMID: 17053790 DOI: 10.1038/nbt1259]
- 77 **Jiang W**, Shi Y, Zhao D, Chen S, Yong J, Zhang J, Qing T, Sun X, Zhang P, Ding M, Li D, Deng H. In vitro derivation of functional insulin-producing cells from human embryonic stem cells. *Cell Res* 2007; **17**: 333-344 [PMID: 17426693 DOI: 10.1038/cr.2007.28]
- 78 **Zhang D**, Jiang W, Liu M, Sui X, Yin X, Chen S, Shi Y, Deng H. Highly efficient differentiation of human ES cells and iPS cells into mature pancreatic insulin-producing cells. *Cell Res* 2009; **19**: 429-438 [PMID: 19255591 DOI: 10.1038/cr.2009.28]
- 79 **Hrvatin S**, O'Donnell CW, Deng F, Millman JR, Pagliuca FW, DiIorio P, Rezanian A, Gifford DK, Melton DA. Differentiated human stem cells resemble fetal, not adult,  $\beta$  cells. *Proc Natl Acad Sci USA* 2014; **111**: 3038-3043 [PMID: 24516164 DOI: 10.1073/pnas.1400709111]
- 80 **Xie R**, Everett LJ, Lim HW, Patel NA, Schug J, Kroon E, Kelly OG, Wang A, D'Amour KA, Robins AJ, Won KJ, Kaestner KH, Sander M. Dynamic chromatin remodeling mediated by polycomb proteins orchestrates pancreatic differentiation of human embryonic stem cells. *Cell Stem Cell* 2013; **12**: 224-237 [PMID: 23318056 DOI: 10.1016/j.stem.2012.11.023]
- 81 **Kroon E**, Martinson LA, Kadoya K, Bang AG, Kelly OG, Eliazar S, Young H, Richardson M, Smart NG, Cunningham J, Agulnick AD, D'Amour KA, Carpenter MK, Baetge EE. Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. *Nat Biotechnol* 2008; **26**: 443-452 [PMID: 18288110 DOI: 10.1038/nbt1393]
- 82 **Kelly OG**, Chan MY, Martinson LA, Kadoya K, Ostertag TM, Ross KG, Richardson M, Carpenter MK, D'Amour KA, Kroon E, Moorman M, Baetge EE, Bang AG. Cell-surface markers for the isolation of pancreatic cell types derived from human embryonic stem cells. *Nat Biotechnol* 2011; **29**: 750-756 [PMID: 21804561 DOI: 10.1038/nbt.1931]
- 83 **Chetty S**, Pagliuca FW, Honore C, Kweudjeu A, Rezanian A, Melton DA. A simple tool to improve pluripotent stem cell differentiation. *Nat Methods* 2013; **10**: 553-556 [PMID: 23584186 DOI: 10.1038/nmeth.2442]
- 84 **Basford CL**, Prentice KJ, Hardy AB, Sarangi F, Micallef SJ, Li X, Guo Q, Elefany AG, Stanley EG, Keller G, Allister EM, Nostro MC, Wheeler MB. The functional and molecular characterisation of human embryonic stem cell-derived insulin-positive cells compared with adult pancreatic beta cells. *Diabetologia* 2012; **55**: 358-371 [PMID: 22075915 DOI: 10.1007/s00125-011-2335-x]
- 85 **Melton DA**. Using stem cells to study and possibly treat type 1 diabetes. *Philos Trans R Soc Lond B Biol Sci* 2011; **366**: 2307-2311 [PMID: 21727136 DOI: 10.1098/rstb.2011.0019]
- 86 **Pagliuca FW**, Melton DA. How to make a functional  $\beta$ -cell. *Development* 2013; **140**: 2472-2483 [PMID: 23715541 DOI: 10.1242/dev.093187]
- 87 **Pagliuca FW**, Millman JR, Gürtler M, Segel M, Van Dervort A, Ryu JH, Peterson QP, Greiner D, Melton DA. Generation of functional human pancreatic  $\beta$  cells in vitro. *Cell* 2014; **159**: 428-439 [PMID: 25303535 DOI: 10.1016/j.cell.2014.09.040]
- 88 **Rezanian A**, Bruin JE, Arora P, Rubin A, Batushansky I, Asadi A, O'Dwyer S, Quiskamp N, Mojibian M, Albrecht T, Yang YH, Johnson JD, Kieffer TJ. Reversal of diabetes with insulin-producing cells derived in vitro from human pluripotent stem cells. *Nat Biotechnol* 2014; **32**: 1121-1133 [PMID: 25211370 DOI: 10.1038/nbt.3033]
- 89 **Tan G**, Elefany AG, Stanley EG.  $\beta$ -cell regeneration and differentiation: how close are we to the 'holy grail'? *J Mol Endocrinol* 2014; **53**: R119-R129 [PMID: 25385843 DOI: 10.1530/JME-14-0188]
- 90 **Hebrok M**. Generating  $\beta$  cells from stem cells-the story so far. *Cold Spring Harb Perspect Med* 2012; **2**: a007674 [PMID: 22675664 DOI: 10.1101/cshperspect.a007674]
- 91 **Kushner JA**, MacDonald PE, Atkinson MA. Stem cells to insulin secreting cells: two steps forward and now a time to pause? *Cell Stem Cell* 2014; **15**: 535-536 [PMID: 25517460 DOI: 10.1016/j.stem.2014.10.012]
- 92 **Kojima H**, Fujimiya M, Terashima T, Kimura H, Chan L. Extraprostatic proinsulin/insulin-expressing cells in diabetes mellitus: is history repeating itself? *Endocr J* 2006; **53**: 715-722 [PMID: 16960402 DOI: 10.1507/endocrj.KR-84]
- 93 **Fan Y**, Rudert WA, Grupillo M, He J, Sisino G, Trucco M. Thymus-specific deletion of insulin induces autoimmune diabetes. *EMBO J* 2009; **28**: 2812-2824 [PMID: 19680229 DOI: 10.1038/emboj.2009.212]
- 94 **Devaskar SU**, Giddings SJ, Rajakumar PA, Carnaghi LR, Menon RK, Zahm DS. Insulin gene expression and insulin synthesis in mammalian neuronal cells. *J Biol Chem* 1994; **269**: 8445-8454 [PMID: 8132571]
- 95 **Kojima H**, Fujimiya M, Matsumura K, Nakahara T, Hara M, Chan L. Extraprostatic insulin-producing cells in multiple organs in diabetes. *Proc Natl Acad Sci USA* 2004; **101**: 2458-2463 [PMID: 14983031 DOI: 10.1073/pnas.0308690100]
- 96 **Kasik JW**, Lu C, Menon RK. The expanding insulin family: structural, genomic, and functional considerations. *Pediatr Diabetes* 2000; **1**: 169-177 [PMID: 15016228 DOI: 10.1034/j.1399-5448.2000.010308.x]
- 97 **Lu C**, Lam HN, Menon RK. New members of the insulin family: regulators of metabolism, growth and now ... reproduction. *Pediatr Res* 2005; **57**: 70R-73R [PMID: 15817502 DOI: 10.1203/01.PDR.0000159573.55187.CA]
- 98 **Bradbury A**, Plückthun A. Reproducibility: Standardize antibodies used in research. *Nature* 2015; **518**: 27-29 [PMID: 25652980 DOI: 10.1038/518027a]
- 99 **Baker M**. Reproducibility crisis: Blame it on the antibodies. *Nature* 2015; **521**: 274-276 [PMID: 25993940 DOI: 10.1038/521274a]
- 100 **Pozzilli P**, Battelino T, Danne T, Hovorka R, Jarosz-Chobot P, Renard E. Continuous subcutaneous insulin infusion in diabetes: patient populations, safety, efficacy, and pharmacoeconomics. *Diabetes Metab Res Rev* 2016; **32**: 21-39 [PMID: 25865292 DOI: 10.1002/dmrr.2653]
- 101 **Battelino T**, Liabat S, Veeze HJ, Castañeda J, Arrieta A, Cohen O. Routine use of continuous glucose monitoring in 10 501 people with diabetes mellitus. *Diabet Med* 2015; **32**: 1568-1574 [PMID: 26042926 DOI: 10.1111/dme.12825]
- 102 **Lee AS**, Tang C, Rao MS, Weissman IL, Wu JC. Tumorigenicity as a clinical hurdle for pluripotent stem cell therapies. *Nat Med* 2013; **19**: 998-1004 [PMID: 23921754 DOI: 10.1038/nm.3267]

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## Evidence based review of type 2 diabetes prevention and management in low and middle income countries

Aimee Afable, Nidhi Shree Karingula

Aimee Afable, Nidhi Shree Karingula, Department of Community Health Sciences, SUNY Downstate School of Public Health, New York, NY 11203, United States

**Author contributions:** Afable A conceptualized the design of the study and led writing of paper; under the guidance of Afable A and Karingula NS conducted the literature review and documented the process; Karingula NS also assisted in the synthesis, analysis and writing up of findings.

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**Correspondence to:** Aimee Afable, PhD, MPH, Assistant Professor, Department of Community Health Sciences, SUNY Downstate School of Public Health, 450 Clarkson Avenue, Box 43, Brooklyn, New York, NY 11203, United States. [aimee.afable-munsuz@downstate.edu](mailto:aimee.afable-munsuz@downstate.edu)  
Telephone: +1-718-2706397  
Fax: +1-718-2215157

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### Abstract

**AIM:** To identify the newest approaches to type 2 diabetes (T2DM) prevention and control in the developing

world context.

**METHODS:** We conducted a systematic review of published studies of diabetes prevention and control programs in low and middle-income countries, as defined by the World Bank. We searched PubMed using Medical Subject Headings terms. Studies needed to satisfy four criteria: (1) Must be experimental; (2) Must include patients with T2DM or focusing on prevention of T2DM; (3) Must have a lifestyle intervention component; (4) Must be written in English; and (5) Must have measurable outcomes related to diabetes.

**RESULTS:** A total of 66 studies from 20 developing countries were gathered with publication dates through September 2014. India contributed the largest number of trials (11/66). Of the total 66 studies reviewed, all but 3 studies reported evidence of favorable outcomes in the prevention and control of type 2 diabetes. The overwhelming majority of studies reported on diabetes management (56/66), and among these more than half were structured lifestyle education programs. The evidence suggests that lifestyle education led by allied health professionals (nurses, pharmacists) were as effective as those led by physicians or a team of clinicians. The remaining diabetes management interventions focused on diet or exercise, but the evidence to recommend one approach over another was weak.

**CONCLUSION:** Large experimental diabetes prevention/control studies of dietary and exercise interventions are lacking particularly those that consider quality rather than quantity of carbohydrates and alternative exercise.

**Key words:** Diabetes prevention and control; Low-income countries; Middle-income countries; Intervention research; Systematic reviews

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**Core tip:** We conducted a systematic review of published

efficacy studies of diabetes prevention and control programs in low and middle-income countries. A total of 66 studies from 20 countries were gathered, based on our selection criteria. Of the 66 studies, all but 3 reported evidence of efficacy. Structured lifestyle education programs were the most common strategies. There was also a diverse range of dietary and exercise approaches. However, large experimental studies of their efficacy, particularly with regard to studies comparing alternative exercise to aerobic and quality of carbohydrates to quantity, are lacking.

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## INTRODUCTION

Diabetes leads to both premature death and complications such as blindness, amputations, renal disease, and cardiovascular diseases<sup>[1]</sup>. It is well known that risk factors for diabetes such as physical activity and diet are modifiable and can possibly be reversed with adjustments in lifestyle; there is an opportunity to intervene and prevent or delay onset of diabetes.

Diabetes often disproportionately affects low- and middle-income countries. More than 382 million people (8.3%) in this world are suffering from diabetes and it is projected to rise to more than 592 million by 2035<sup>[2]</sup>. China and India lead in the number of cases worldwide. For example, it is estimated that 98.4 million adults in China and 65.1 million in India have diabetes<sup>[2]</sup>. China now has the largest epidemic worldwide and recent study suggest that diabetes prevalence in China has surpassed the United States with 11.6% of Chinese adults having diabetes<sup>[3]</sup>.

Diabetes growth worldwide has been attributed to global secular shifts in lifestyles that result from upward social mobility and rapid urbanization<sup>[4-8]</sup>. Intra-country migrants who move from rural to urban areas, or who transition from poverty to affluence, for example, can take on more sedentary jobs, markedly different from their former labor-intensive work and adopt less healthy diets<sup>[9,10]</sup>. This shift to a more sedentary lifestyle and greater consumption of processed foods and total energy intake is common in middle-income countries undergoing rapid urbanization, a process that has been labeled the "nutrition transition"<sup>[11,12]</sup>.

Further, diabetes is now affecting younger and middle-aged adults who are at the peak of their economic productivity<sup>[13-16]</sup>. Costs associated with the care and management of diabetes worldwide is significant. People with diabetes have more outpatient visits, use more medications, have a higher probability of being hospitalized, and are more likely to require emergency and long-term care than

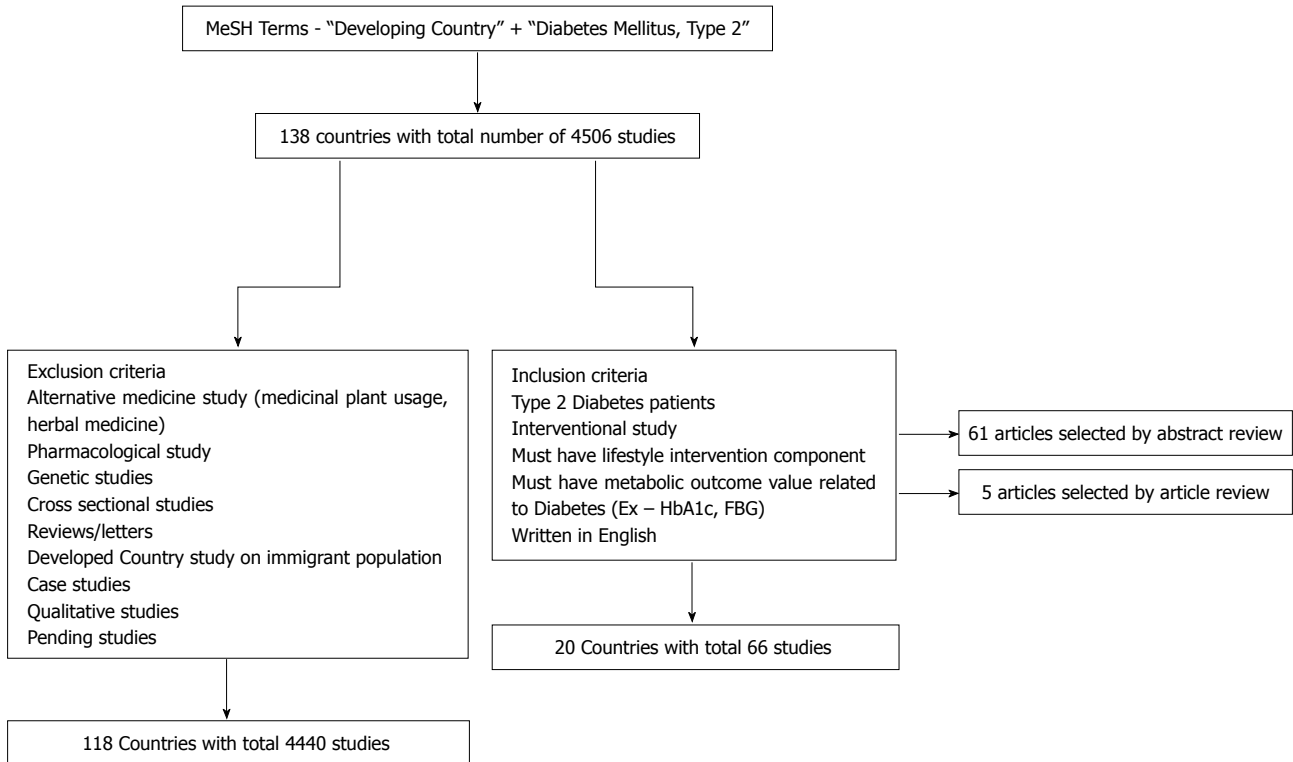
people without the disease<sup>[17,18]</sup>. In the United States for example, chronic disease management and diabetes in particular is a major driver of healthcare costs<sup>[19]</sup>. In the United States, people with diabetes have 2-3 times health care costs compared to those without diabetes<sup>[18]</sup>. According to American Diabetes Association, total costs of diagnosed diabetes have risen to \$245 billion in 2012 from \$174 billion in 2007. United States adults with diagnosed diabetes incur average medical expenditures of about \$13700 per year, of which about \$7900 is attributed to diabetes<sup>[18]</sup>.

Research on the efficacy of diabetes prevention and control efforts have been concentrated in the United States and Europe<sup>[15,20,21]</sup>, but the burden of disease is felt around the globe. By limiting research to high-income countries we may neglect the potential for high- and low-income countries to learn from each other, and for leveraging global resources in the development of more cost-effective strategies<sup>[14]</sup>. The National Institutes of Health-funded randomized controlled trial in the United States, the Diabetes Prevention Program (DPP), reported a 58% reduction in risk of developing type 2 diabetes through intensive lifestyle intervention among participants who were overweight and had prediabetes<sup>[22]</sup>. United States and global efforts are underway to translate this trial to populations who are disproportionately affected<sup>[20,21]</sup>. A meta-analysis of DPP translations in the United States highlights significant heterogeneity in approaches to translation including the use of allied health professionals vs lay community members to deliver lifestyle education; with regard to the number of educational sessions and the integration of technology<sup>[20]</sup>. Overall, Ali *et al*<sup>[20]</sup> found an adjusted pooled mean weight loss of 4 pounds. The authors conclude that there was no consistent pattern with regard to which type of DPP translation was more effective. However, they argued that there was no evidence to suggest that interventions that used lay members as opposed to allied health professionals were less effective; they propose that the use of lay health members to deliver lifestyle education are potentially more cost-effective than those that use allied health professionals.

Building on the Ali *et al*<sup>[20]</sup> review, this paper aims to identify the newest approaches to diabetes prevention and control in the developing world context, and highlight the unique considerations and challenges when working to prevent and manage chronic disease from a global perspective. The specific objectives of our review are the following (1) to evaluate whether interventions that are similar to DPP in the developing world are effective; (2) identify interventions that are substantively different from the DPP (with regard to intervention components); and (3) among this latter group, evaluate whether there is evidence of efficacy.

## MATERIALS AND METHODS

According to the World Bank New Country Classifications, low and middle income countries are considered developing economies<sup>[23]</sup>. A low income country is defined as having



**Figure 1** Systematic search methodology and results.

a gross national income (GNI) of \$1035 or less, whereas a middle income country is defined as having a GNI between \$1036 and \$12615<sup>[1]</sup>. Currently, 138 countries in the world are considered to be developing economies. Using this list of countries, a systematic search through PubMed was conducted. Using Medical Subject Headings (MeSH terms), studies in the developing world on type 2 diabetes (T2DM) were obtained. For example, to search through studies in Algeria, the following terms were used - "Diabetes Mellitus, Type 2"(Mesh) AND "Algeria"(Mesh). Thus, searching country by country, 4506 studies involving type 2 diabetes were gathered from 97 developing countries during two search phases: September 2013 to December 2013 and an updated search during August 2014 to September 2014.

These studies were subsequently manually sorted using a pre-determined inclusion and exclusion criteria. The study needed to satisfy four criteria: (1) Must be experimental; (2) Must include patients with T2DM or focusing on prevention of T2DM; (3) Must have a lifestyle intervention component; (4) Must be written in English; and (5) Must have measurable outcomes related to diabetes. Specifically, "Lifestyle Intervention" was defined as any intervention that involved an exercise, dietary, behavioral change element modification. The behavioral change also included counseling on self-management, smoking cessation or stress management. Additionally, a measurable outcome value was defined as any outcome measure of diabetes or risk factor for diabetes such as hemoglobin A1c (HbA1c), fasting plasma glucose, blood glucose and insulin levels, and body mass index/obesity.

Using the above mentioned inclusion criteria, the

studies were manually reviewed and filtered. Studies were excluded if they were: (1) Non-experimental/observational; (2) Pharmacological Studies; (3) Reviews; (4) Evaluated herbal medicines only; (5) Genetic Studies (studies that looked at specific gene variations in diabetic patients); (6) Studies that were conducted on immigrant populations in developed countries; and (7) Pending Studies. Based on the inclusion criteria, the studies were first filtered through abstract review and article review. Sixty-one articles were selected by abstract review and 5 articles were selected from article review. Therefore, a total of 66 studies from 20 developing countries were gathered after applying these inclusion and 4450 studies from 119 countries were excluded based on the exclusion criteria. Six pending studies were also collected and separately recorded. Figure 1 gives a visual reference of the search methodology.

Finally to address the primary objectives of the review paper, studies were classified into three categories: (1) Those most similar to the DPP and thus had a primary emphasis on lifestyle education/counseling delivered by allied health professionals or lay members of the community where dietary and exercise modification were recommended but not provided; (2) Intervention studies where structured dietary plans and exercise/activity modification were the main components; and (3) Any intervention that integrated some form of technology (texting, website, telephone, glucose monitor, etc.). We assess evidence of efficacy<sup>[24]</sup>. We defined a study as having evidence of efficacy if there were statistically significant differences: (1) Between baseline and follow-up



in experimental group; or (2) Between experimental and control groups in any of the primary outcomes reported.

## RESULTS

### **Diabetes management: DPP adaptations**

As shown in Table 1 nature of the intervention and study methodology varied widely among the studies reviewed. For example, among the 29 interventions that were most similar to DPP that were evaluated, 15 were randomized control trials (RCTs) and the remainder utilized quasi-experimental designs to evaluate the efficacy of the interventions. Sample size also varied in this group of studies. For example, the Turkish RCT by Mollaoğlu *et al.*<sup>[25]</sup> 2009 had a sample size of 50; in contrast the RCT conduct in Bulgaria by Tankova, 2004 had a sample size of 560<sup>[26]</sup>. Among all study countries, Brazil and Thailand contributed the largest number of trials (5 and 4 respectively).

Among all 29 interventions, the follow-up period for outcome measurement also varied with a minimum of 2 mo follow-up<sup>[25]</sup> to a maximum follow-up period of 7 years<sup>[27]</sup>. Of all 29 studies, the Iranian study (Sarrafzadegan *et al.*<sup>[27]</sup>, 2013) was quite impressive. They evaluated the impact of a community-based lifestyle education mass-media intervention in a population of 9032 adults and during a follow-up period of 7 years and found significant declines abdominal obesity, hypertension and lipid biomarkers; however, there were not significant changes in blood glucose and diabetes prevalence<sup>[27]</sup>.

Of the 29 interventions, all but 2 studies found significant improvements in diabetes related outcomes. Eight interventions delivered intensive lifestyle modification sessions by a physician or team of clinicians (*e.g.*, nutritionist, nurse, physician) and all but 1 study<sup>[28]</sup> found significant improvements in glycemic control in the experimental groups<sup>[26,29-34]</sup>. Twelve of the interventions were nurse-led, and all but 1 study<sup>[25]</sup> found significant improvement in glycemic control and/or significant decline in body mass index (BMI)<sup>[33,35-44]</sup>. Eight of the interventions were pharmacist-led and focused primarily on medication consultation (adherence and adjustments in dosage) and in some cases also included lifestyle education; all found significant improvement in glycemic control in experimental group<sup>[45-52]</sup>. Only one of the studies (in Jamaica) utilized lay health workers to deliver lifestyle education, and found significant decline in HbA1c levels in the experimental group<sup>[53]</sup>.

### **Diabetes management: Diet and exercise as main component**

As shown in Table 2, India contributed the largest number of trials with 6 total. Among all 18 studies, the nature of the intervention and study methodology varied widely. All 18 studies found significant improvements in diabetes related outcomes. Study designs varied and length of follow-up ranged from 2 wk (Chaiapanont<sup>[54]</sup>, 2008) to 3 years (Oli *et al.*<sup>[55]</sup>, 1984). Among the 18 interventions, 7

focused exclusively on modifying diet with an emphasis on increasing fiber intake, introduction of low glycemic foods or variation in carbohydrate content<sup>[55-60]</sup>; these interventions took place in Brazil, India, Nigeria, Thailand and Mexico. It is notable that all of these studies were pilot studies with very small sample sizes ranging from 10 (Komindr *et al.*<sup>[58]</sup>, 2001) to 160 (Oli *et al.*<sup>[55]</sup>, 1984). In all the studies that used single group designs, all reported significant improvements in blood glucose control or cardiovascular risk factors. Oli *et al.*<sup>[55]</sup>, one of the largest trials, investigated the noteworthy question of whether diabetic patients can maintain blood glucose control with a high carbohydrate diet, consisting of readily available Nigerian foods and found excellent/good blood glucose control in over half of their patients (mean fasting blood glucose of 7-8 mmol/L or less). In the only study that used an RCT crossover design in Mexico ( $n = 14$ ), there was HbA1c was significantly lower during the low-glycemic index period relative to the high-glycemic index period<sup>[59]</sup>. In addition one small Indian cross-over study examined the effect of camel milk on glycemic control and insulin sensitivity and found lower HbA1c in the diabetic group that drank camel milk (and deterioration in glycemic control when they drank cow milk)<sup>[61]</sup>.

Ten of the 18 studies focused exclusively on a structured physical activity program. These studies investigated the efficacy of some form of structured exercise including aerobic walking/exercise (in some cases these studies also involved nutrition counseling but no structured diet)<sup>[62-66]</sup>, progressive resistance training (PRT)<sup>[67,68]</sup>, mixed aerobic/PRT exercise program<sup>[69]</sup>, and yoga/breathing/sitting/relaxation program<sup>[54,70]</sup>. All 10 studies found improvements in blood glucose control<sup>[54,63-69]</sup>, BMI<sup>[70]</sup> or general well-being<sup>[62]</sup>. Among these 10, 4 were RCTs<sup>[62-64,68]</sup>. It is notable that the RCT conducted by Arora *et al.*<sup>[68]</sup>, 2009 in India found significant decreases in HbA1c levels in PRT group, which was comparable to the decrease in HbA1c level in the aerobic exercise group. Similarly, the largest trial of the structured exercise studies, which was conducted in South Africa, found significant decreases in HbA1c levels in both relaxation group and its comparison the aerobic exercise group<sup>[63]</sup>. Only 1 study in China involved both structured exercise and dietary change components; this was an RCT and found significant improvements in glycemic control in experimental group<sup>[71]</sup>.

### **Diabetes management: Technology assisted interventions**

In total we identified 9 interventions that integrated some form of technology including glucose monitoring systems, telehealth, multi-media and short message service (SMS) texting (Table 3). Three studies from Bangladesh, Bulgaria, and Malaysia evaluated the efficacy of home/self-monitoring of glucose (and also integrated health/lifestyle education); all three found significant improvements in glycemic control<sup>[72-74]</sup>. It is notable that both Kempf *et al.*<sup>[73]</sup> and Ismail *et al.*<sup>[74]</sup>

**Table 1 Diabetes management: Diabetes Prevention Program like interventions (allied health professionals and lay member facilitated)**

Country	Ref.	Objective	Study design	Sample size/ characteristics	Components of intervention	Measurements	Outcome measures	Conclusion
Argentina	Gagliardino <i>et al</i> <sup>[29]</sup>	To evaluate effect of combined physician and/or patient education and effect of system interventions (100% coverage of medications, formalized data collection)	Randomized 2 × 2 design trial	<i>n</i> = 468, 117 in control group (g1), 117 in physician education group (g2), 117 in patient education group (g3), 117 in physician and patient education group (g4), T2DM for at least 2 yr, age b/w25 and 75 yr	For T2DM pts - 90-120 min weekly teaching units For physicians - 25 structured module interactive course	HbA1c, BMI, FBG	0, 6, 12, 18, 24, 30, 36, 42 mo	HbA1c decreased from 4 mmol/mol to 10 mmol/mol ( <i>P</i> < 0.05), with the largest decrease being in g4 (physician and patient education group)
Brazil	Cezaretto <i>et al</i> <sup>[30]</sup>	To evaluate effect of interdisciplinary intervention program	Two group randomized longitudinal	<i>n</i> = 135, 60 in traditional group, 75 in intensive group, high risk individuals for T2DM between ages 18 and 79	Intensive Intervention group - 2 h group sessions from 4 sessions in month 1 to 2 sessions in month 2 and 1 monthly sessions until 9 mo, print materials, telephone calls, interdisciplinary team included endocrinologist, psychologist, nutritionist, and physical educator	FBS, BMI, post 0 and 9 load plasma glucose	0 and 9 mo	Intensive intervention group decreased fasting plasma glucose from 98.9 to 95.3 ( <i>P</i> < 0.001), while the traditional intervention group was not significant. Intensive intervention group BMI decreased from 31.7 to 30.9 ( <i>P</i> < 0.001) while the traditional intervention group BMI decreased from 29.9 to 29.1 ( <i>P</i> < 0.001)
Brazil	Chaves-Fonseca <i>et al</i> <sup>[31]</sup>	To evaluate effectiveness of “staged diabetes management” protocol	RCT	<i>n</i> = 113, 47 in control group, 66 in intervention group > 30 yr old, T2DM	SDM protocol (as developed International diabetes center) with doctor, nurse, pharmacist and health technicians	HbA1c, random glucose	0, 12 and 18 mo	Random glucose decreased from 12.7 to 10.5 ( <i>P</i> = 0.004) and HbA1c decreased from 9.2 to 7.7 ( <i>P</i> < 0.001) in intervention group, while there was no significant change in intervention group
Brazil	Mourão <i>et al</i> <sup>[49]</sup>	To evaluate effectiveness of pharmaceutical care program	RCT	<i>n</i> = 100, 50 in control and interventional, > 18 yr, HbA1c > 7%, post prandial capillary glucose > 180 mg/dL, T2DM	Two research pharmacists conducted education on drug therapy problems, medication adherence	HbA1c, fasting blood glucose	0 and 6 mo	HbA1c decreased -0.6% and fasting blood glucose decreased -21.4 mg/dL in intervention group ( <i>P</i> = 0.001)
Brazil	Correr <i>et al</i> <sup>[50]</sup>	To evaluate effect of pharmacotherapy follow up	RCT	<i>n</i> = 96, 50 in intervention and 46 in control, > 30 yr old, diagnosed T2DM, oral meds or insulin use	Monthly visit with pharmacist for education, suggestion in changes of medication and dosage changes	HbA1c, fasting capillary glycemia	0 and 12 mo	Relative to the control group, the intervention group exhibited greater glycosylated haemoglobin (HbA1) reduction [-2.2% (95%CI, -2.8%:-1.6%) <i>vs</i> -0.3 (95%CI, -0.8-0.2); <i>P</i> < 0.001] and greater fasting capillary glycaemia reduction [-20.1 mg/dL (95%CI, -31.9 mg/dL: -8.3 mg/dL) <i>vs</i> 4.3 mg/dL (95%CI, -13.4 mg/dL: 22.2 mg/dL); <i>P</i> = 0.022]

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Brazil	Borges <i>et al</i> <sup>[51]</sup>	To evaluate effect of pharmaceutical care	Two group experimental	<i>n</i> = 71, 31 in control group and 40 in intervention group, > 18 yr old, T2DM	Individual visit with pharmacist monthly, patient education, dosage adjustment	Fasting glycemia, HbA1c	0 and 12 mo	A significant reduction in the levels of glycosylated haemoglobin was detected in patients in the pharmaceutical care group, and an average increase was observed in the control group
Bulgaria	Petkova <i>et al</i> <sup>[52]</sup>	To evaluate effectiveness of educational programme by pharmacists	Single group	<i>n</i> = 24, 31-75 yr, diagnosed T2DM	Educational Sessions with five teaching units over one month	Blood glucose levels, frequency of hypoglycemic Incidents	0, 1, 3 and 6 mo	Education of diabetic patients by pharmacists can decrease the economic cost of T2DM management and benefit patients. Blood glucose levels decreased from 8 to 7.2 mmol/L ( <i>P</i> < 0.05)
Bulgaria	Tankova <i>et al</i> <sup>[26]</sup>	To evaluate effectiveness of a teaching program 1 to 2 yr after implementation	RCT	<i>n</i> = 560, 319 in experimental group, 241 in control group, Insulin treated T1 + 2DM	Geneva-Düsseldorf Education Session Model (consists of lessons on DM, practical training on self-control, injection techniques, preparing meals, construction of menu, physical exercise) education is conducted by team of doctors, nurses and rehab therapist using interactive approach	HbA1c, Well-being as measured by 22-item questionnaire	0, 12 and 24 mo	Structured teaching education program improves patient's well being. Improvement in glycemic control of educated patients as compared to control group ( <i>P</i> < 0.01) and increase in overall wellbeing ( <i>P</i> < 0.001)
Cameroon	Kengne <i>et al</i> <sup>[35]</sup>	To evaluate effectiveness of nurse-led care	Population based sample participants referred to either one of the 2 rural clinics or one of the 3 urban clinics	<i>n</i> = 225, 39 in rural clinic and 186 in urban, T2DM	Education, clinic visits, monitoring, follow-up	Mean fasting capillary glucose	0 and visit 6 (varied over 1110 patient-months)	Difference in mean levels of fasting glucose between baseline and final visit was 1.6 mmol/L ( <i>P</i> < 0.001)
Cameroon	Labhardt <i>et al</i> <sup>[36]</sup>	To evaluate effectiveness of non-physician clinician facility care	Included all of the 75 clinics in central region of cameroon	<i>n</i> = 79, T2DM	Protocol-drive care by non-physician clinicians (nurses), diet and lifestyle education	Fasting Plasma glucose	0 and 2 yr	Fasting plasma glucose decreased -7.8 mmol/L ( <i>P</i> < 0.001)
China	Liu <i>et al</i> <sup>[28]</sup>	To evaluate effectiveness of group visit and self management model	RCT	<i>n</i> = 176, 98 in intervention group and 78 in control group, T2DM, between 35-80 yr	12 1.5 h sessions on self management education, one-on-one visits with health care providers, including nurse, general practitioner and diabetes specialist	BMI, SBP, DBP	0 and 12 mo	No significant changes in BMI or DBP in either group, significant change in SBP in intervention group of 1.48 ( <i>P</i> = 0.04). Larger studies need to be done to determined effects of group visits on blood glucose and other metabolic parameters
China	Chen <i>et al</i> <sup>[119]</sup>	To evaluate effectiveness of nurse diabetes intervention	Quasi-experiment, pre and post-test	<i>n</i> = 150, 75 in each control and case groups, > 65 yr, diagnosed T2DM, HbA1c > 8.5%	Self-management education with visits lasting 30 min each, telephone follow-up two weekly	BP, HbA1c, Weight	0 and 3 mo	Nurse-led education and consultation is effective in improving management in T2DM patients. HbA1c in case group changed -0.8% ( <i>P</i> < 0.001) while the control group had no significant change

Iran	Sarrafadeghan <i>et al</i> <sup>[27]</sup>	To evaluate effect of comprehensive, community based healthy lifestyle program on cardiometabolic risk factors	Multi-stage cluster, 2 areas	$n = 9032, 4179$ in intervention area, 4853 in reference area, general population (htn, metabolic syndrome, diabetes, cardiac disease pts)	Public education through mass media, healthy nutrition, increased physical activity, tobacco control and coping with stress	Cholesterol, abdominal obesity, fasting blood glucose	0, 7 yr	Mean fasting blood glucose increased, but prevalence of abdominal obesity, htn, hypercholesterolemia and hypertriglyceridemia decreased significantly in intervention area ( $P < 0.05$ ), no significant change in prevalence of diabetes
Iran	Farsaei <i>et al</i> <sup>[47]</sup>	To evaluate effectiveness of pharmacist-led education program	RCT	$n = 172$ , diagnosed T2DM, HbA1c > 7%	Two educational sessions followed by weekly phone calls and appointments, medication consultation	FBS, HbA1c	0 and 3 mo	There is improvement in diabetes management by involvement of pharmacist in multidisciplinary health care team. HbA1c and FBS (-1.7% and -30.8 mg/dL) were decreased in intervention group ( $P < 0.001$ )
Jamaica	Less <i>et al</i> <sup>[33]</sup>	To evaluate effectiveness of involvement of LDFs	Two group experimental	$n = 293, 158$ in intervention group and 135 in control group, 25-75 yr, diagnosed T2DM	Educational Sessions during 3 monthly visits, self-monitoring forms	HbA1c, BMI	0 and 6 mo	Patient education by LDFs improved glycemic control of T2DM patients. HbA1c reduced from 0.6% in intervention group ( $P < 0.001$ ) while comparison group had an increase of 0.6% ( $P < 0.001$ )
Jordan	Jarab <i>et al</i> <sup>[48]</sup>	To evaluate effectiveness of pharmacist-led pharmaceutical care intervention program	RCT	$n = 171, 85$ in intervention group and 86 in control group, > 18 yr, diagnosed T2DM for at least 1 yr, HbA1c > 7.1%	Medication consultation, lifestyle education, follow-up calls 8 weekly	FBG, HbA1c, BMI, Lipid Panel, BP	0 and 6 mo	Pharmacist-led pharmaceutical care led to an improvement in glycemic parameters. Intervention group had a mean reduction of 0.8% HbA1c versus a mean increase of 0.1% in the usual care group ( $P = 0.019$ ). FBG in intervention group had a reduction of 2.3 mmol/L and the intervention group showed an increase of 0.9 mmol/L ( $P = 0.014$ )
Malaysia	Tan <i>et al</i> <sup>[33]</sup>	To evaluate effectiveness of structured diabetes education program	Single blind RCT	$n = 164, 82$ in control and intervention group, > 18 yr, diagnosed T1 + T2DM, HbA1c > 7%	Educational sessions once a month for 3 mo self-care practices, individual counseling with nurse and physician	HbA1c, SMBG frequency	0, 1, 2 and 3 mo	A self-management diabetes education program improves the well-being of diabetic patients. Intervention group had lower HbA1c than control group by the end of study (intervention group - $P < 0.001$ , hbac decreased $8.75 \pm 1.75$ ; control group $9.67 \pm 2.01$ )
Mexico	Gallegos <i>et al</i> <sup>[39]</sup>	To evaluate effectiveness of nurse-led education	Two group quasi-experiment	$n = 45, 25$ in experimental group and 20 in control group, diagnosed T2DM	6 Educational sessions lasting 90 min each, 20 individual counseling sessions lasting 30 to 90 min throughout 50 wk	HbA1c, psychological adaptation, diabetes care skills	0, 3, 6, 9 and 12 mo	Counseling and education model is an effective intervention to improve metabolic control in T2DM patients. HbA1c decreased from 10.36 at baseline to 8.04 ( $P = 0.000$ ) while comparison group HbA1c levels changed from 9.44 to 9.77
Samoa	DePue <i>et al</i> <sup>[37]</sup>	To evaluate effectiveness of nurse-community health workers team intervention for diabetes management	Cluster rct	$n = 243, 140$ in usual care group, 104 in intervention group, > 18 yr, T2DM	Group visits and individual visits based on risk of patients	HbA1c	0 and 12 mo	Mean HbA1c was significantly lower among CHW participants, compared with usual care, after



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South Africa	Price <i>et al</i> <sup>[40]</sup>	To determine long-term glycemic outcome of a structured nurse-led care	Single group, single center	<i>n</i> = 80, T2DM	Nurse led drug titration, structured empowerment based diabetes education	HbA1c, BMI	0, 6 mo, 18 mo, 2 yr, 4 yr	BMI at 6 and 18 mo was significantly higher than at baseline (both <i>P</i> < 0.01), but the 48 mo value was not significantly different from 0 mo. Compared with baseline, HbA1c falls were all significant ( <i>P</i> < 0.001 for 6, 18 and 24 mo and <i>P</i> = 0.015 for 48 mo)
South Africa	Gill <i>et al</i> <sup>[41]</sup>	To determine effectiveness for a nurse-led intervention and education based program	Single group	<i>n</i> = 284, diagnosed T2DM	Self-management education, pictorial based education	HbA1c, BMI	0, 6 and 18 mo	Nurse-led protocol and education based intervention improve glycemic parameters in diabetic patients. HbA1c was 11.6% at baseline, but improved to 7.7% at 18 mo
Thailand	Wattana <i>et al</i> <sup>[42]</sup>	To evaluate effectiveness of self-diabetes management program	RCT	<i>n</i> = 147, 72 in control and 75 in experimental, > 35 yr, Diagnosed T2DM, FPG > 140 mg	120 min of small group diabetes education class, four 90 min group discussions and two individual home visit sessions by nurse educators	HbA1c, CHD risk, quality of life assessment	0 and 6 mo	A diabetes self-management program is effective in improving metabolic control for T2DM patients. HbA1c change was -0.68 in experimental group ( <i>P</i> = 0.029) and 0.07 in control group
Thailand	Navicharern <i>et al</i> <sup>[43]</sup>	To evaluate effect of multifaceted nurse-coaching intervention	Quasi experiment, 2 group	<i>n</i> = 40, 20 in control and experimental group, T2DM	3 individualized sessions, 2 follow-up phone calls over 12 wk	HbA1c	0 and 3 mo	Mean average of HbA1c of the experimental group was significantly lower than that of the control group [ <i>x</i> (exp) = 7.10, SD = 0.67 <i>vs</i> <i>x</i> (cont) = 7.72, SD = 0.97; <i>P</i> ≤ 0.5]
Thailand	Suppavitipom <i>et al</i> <sup>[45]</sup>	To evaluate effect of pharmacist led intervention	RCT	<i>n</i> = 360, 180 in control and experimental group each (divided into 4 groups), T2DM	Drug counseling, special medical containers, diabetes booklet (in experimental group, 1 group received only drug counseling, 2 <sup>nd</sup> group received drug counseling + special medical containers, 3 <sup>rd</sup> group received drug counseling + diabetes booklet, 4 <sup>th</sup> group received all)	HbA1c, mean fasting glucose	0, 3, 6 mo	Most favorable glycemic outcome was the group that received all of the interventions; mean FPG was reduced from 147.46 ± 36.07 to 125.38 ± 31.12 mg % ( <i>P</i> < 0.000) in 1 <sup>st</sup> visit (3 mo later) and still reducing effect on the 2 <sup>nd</sup> visit (6 mo later) mean FPG from 147.46 ± 36.07 to 130.21
Thailand	Oba <i>et al</i> <sup>[44]</sup>	To evaluate effectiveness of community participation prevention program in diabetes prevention	Single group, pre-post test	<i>n</i> = 160, > 35 yr, BMI > 23 kg/m <sup>2</sup> , waist circumference > 80 cm (women) and > 90 cm (men), FBS 100-125 mg/dL, no baseline diabetes (but at risk patients)	Nutritional education provided by nurse practitioner, fitness schedule in daily exercise log	BMI, SBP, DBP	0, 1, 2, 3 mo	Average mean scores of the BMI ( <i>P</i> < 0.001), SBP ( <i>P</i> < 0.01) and waist circumference ( <i>P</i> < 0.01) among persons who were at risk of DM after the intervention were lower than before intervention
Tunisia	Jenhani <i>et al</i> <sup>[32]</sup>	To evaluate effectiveness of education program on diabetes control	Pre/post-test experiment	<i>n</i> = 87, diagnosed T1 + T2DM, insulin usage	Six education sessions, interactive learning conducted by nurse and general practitioner	HbA1c, BMI, anxiety level	0 and 6 mo	Education program led to an improvement in diabetes control in patients. HbA1c decreased from 8.80% pre intervention to 7.62% ( <i>P</i> < 0.00001)
Turkey	Mollaoğlu <i>et al</i> <sup>[25]</sup>	To evaluate effectiveness of nurse-led planned education	RCT	<i>n</i> = 50, 25 in experimental and control group, 18-65 yr, diagnosed T2DM	3 Educational Sessions 30-40 min each, home visit follow-ups	HbA1c, FBS, lipid panel	0, 1 and 2 mo	Regular, structured, repeated education improves glycemic parameters in T2DM patients, HbA1c and FBS levels changes were not statistically significant
Turkey	Turnacilar <i>et al</i> <sup>[46]</sup>	To evaluate effectiveness of pharmaceutical care program	Prospective longitudinal, cluster	<i>n</i> = 43, T2DM	6 pharmacy visits, drug counseling, weight control importance	Capillary whole blood glucose, BMI	0, 15, 30, 45, 60, 75, 90 d	Mean fasting blood glucose decreased from 167 to 128 mg/dL ( <i>P</i> < 0.001)

Turkey	Kitiş <i>et al</i> <sup>[38]</sup>	To evaluate effect of home monitoring by public health nurse	Quasi experimental, single group, time series	<i>n</i> = 34, T2DM for at least 2 yr	Caloric calculation, exercise recommendations, medication compliance, monitoring blood glucose, education study group, booklets, 1 <sup>st</sup> two months frequency of visits based on patients needs, with 2 <sup>nd</sup> mo, visits every 2 mo	HbA1c, fasting blood glucose, postmeal blood glucose	0 and 6 mo	HbA1c decreased from 7.3% to 6.7% ( <i>P</i> = 0.000), FBG decreased from 186 to 150 ( <i>P</i> = 0.001), postmeal blood glucose decreased from 204 to 156 ( <i>P</i> = 0.000)
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T2DM: Type 2 diabetes mellitus; RCT: Randomized control trial; BMI: Body mass index; HbA1c: Hemoglobin A1c; LDFs: Lay diabetes facilitators.

**Table 2 Diabetes management: Structured dietary change and exercise as main components (not lifestyle education)**

Country	Ref.	Objective	Study design	Sample size/ characteristics	Components of intervention	Measurements	Outcome measures	Conclusion
Brazil	Rodrigues Silva <i>et al</i> <sup>[60]</sup>	To evaluate effect of rice bran fiber diet	Single group	<i>n</i> = 11, 45-60 yr old, controlled diabetes by diet or oral hypoglycemic agents, T1DM + T2DM	1 wk low fiber diet, 2 <sup>nd</sup> week low fiber diet + rice bran, cross over	Mean fasting and post prandial glucose	Daily fasting and postprandial glucose	Mean fasting and postprandial serum glucose levels were reduced, but values of high fiber diet were significantly lower ( <i>P</i> < 0.001) than that of the lower fiber diet
China	Sun <i>et al</i> <sup>[71]</sup>	To evaluate effectiveness of structured integrated intervention program	RCT	<i>n</i> = 150, Intervention group 100 and control group 50, 18-70 yr, BMI > 23 kg/m <sup>2</sup> , T2DM	Nutritional counseling and meal replacement, physical activity instruction, education - monthly group lectures, sample meal plans with applications of meal exchanges and low glycemic index foods	FBG + insulin, HbA1c	0, 3 and 6 mo	An integrated intervention program can achieve improvements in glycemic control. Mean fasting blood glucose values at 24 wk were 7.4 ± 0.2 <i>vs</i> 8.9 ± 0.4 mmol/L ( <i>P</i> < 0.001), intervention <i>vs</i> reference, respectively. No change in HbA1c in reference group, but a -0.8% change observed in intervention group ( <i>P</i> < 0.001)
Costa Rica	Goldhaber-Fiebert <i>et al</i> <sup>[64]</sup>	To evaluate effectiveness of group-centered, community based public health intervention	RCT	<i>n</i> = 61, 33 in intervention group and 28 in control group, diagnosed T2DM	11 weekly nutrition classes 90 min each, triweekly walking physical activity sessions 60 min each	HbA1c, FBG	0 and 3 mo	Community-based, group-centered intervention including nutrition and exercise can improve glycemic control and is economically feasible. Change in FBG in intervention group change -19, control group 16 ( <i>P</i> + 0.048). Change in HbA1c in intervention group -1.8, control group -0.4 ( <i>P</i> = 0.028)
India	Pande <i>et al</i> <sup>[56]</sup>	To investigate effects of low/medium glycemic indexed Indian vegetarian snacks and meal plans on diabetics	Single group experimental	<i>n</i> = 15, 42-58 yr, diagnosed T2DM	Redesigned meal plan focusing on decreasing starches, lipids and increasing fiber	Blood glucose, HbA1c, lipid profile	0, 1, 2, 3 and 4 wk	Significant improvement in metabolic parameters was observed and can be improved if compliance to low/medium GI diet is continued. Blood glucose level of 173.6 mg% at baseline decreased to 137.8 mg% ( <i>P</i> < 0.001), HbA1c of 8% at baseline decreased to 7.1% from baseline ( <i>P</i> < 0.001)

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India	Shenoy <i>et al</i> <sup>[62]</sup>	To evaluate effectiveness of aerobic walking program with pedometer and HRM	RCT	<i>n</i> = 40, 20 in control and 20 in intervention, 40-70 yr, diagnosed T2DM, Not enrolled in any other physical activity program	Timed walking schedule of target 150 min/wk to reach a 50%-70% maximum heart rate, pedometer, HRM	BMI, GWB	0 and 2 mo	Walking with a pedometer and HRM is more effective than walking alone and results in a better wellbeing for T2DM patients
India	Kosuri <i>et al</i> <sup>[70]</sup>	To evaluate effect of yoga on T2DM patients	Single group	<i>n</i> = 35, T2DM	40 d yoga camp with yoga exercises everyday	BMI, general well being	0 and 40 d	BMI decreased from 26.514 to 25.771 ( <i>P</i> < 0.001) and there was also an improvement in total general well being
India	Agrawal <i>et al</i> <sup>[61]</sup>	To investigate effect of camel milk on glycemic control and insulin sensitivity	Two group experimental, crossover	<i>n</i> = 28, T2DM	Cow milk for non diabetic group, camel milk for diabetic group, followed by 3 mo washout period, with switch	FBS, HbA1c, HOMA-IR	0, 1 (run in period), 4 (camel milk period), 5 (washout period), 8 mo (cross over to cow milk)	HbA1c improved due to camel milk consumption ( $8.39 \pm 0.64$ to $7.27\% \pm 0.67\%$ ) whereas deteriorated in the case of cow milk ( $7.36 \pm 0.66$ to $8.26\% \pm 0.60\%$ ) in diabetic group
India	Misra <i>et al</i> <sup>[67]</sup>	To evaluate effectiveness of PRT	Single group	<i>n</i> = 30, diagnosed T2DM	Scheduled PRT training of six muscle groups (two sets, 10 repetitions each), 3 times/wk	HbA1c, blood glucose, lipid profile, BMI	0 and 3 mo	Moderate PRT is effective in improving metabolic parameters in T2DM patients and should be an integral part of their exercise regimen. HbA1c changed 0.54%, ( <i>P</i> < 0.001), fasting blood glucose changed 2.7 mmol/L ( <i>P</i> < 0.001)
India	Arora <i>et al</i> <sup>[68]</sup>	To evaluate effectiveness of PRT compared to aerobic exercise	RCT	<i>n</i> = 30, 10 in supervised PRT, 10 in control group and 10 in aerobic exercise group, 40-70 yr, diagnosed T2DM > 6 mo, inactive lifestyle	Scheduled PRT exercises of 3 sets of 10 repetitions for 2 times per week, aerobic exercise of walking 30 min/d three times a week	HbA1c, BP, BMI, lipid profile, GWB	0 and 2 mo	Metabolic parameters in T2DM patients improved more with PRT compared to aerobic exercise. HbA1c levels decreased ( <i>P</i> < 0.05) both in the PRT group (7.57% to 6.23%) and in Aerobic Exercise group (8.11% to 6.66%)
Iran	Yazdanpanah <i>et al</i> <sup>[66]</sup>	To evaluate effectiveness of community based participatory diabetes care program	Single group, CBPR	<i>n</i> = 320, 30-65 yr, diagnosed T2DM, impaired fasting glucose	Nutrition classes 90 min each 2 d per week for 4 wk, structured physical activity 60 min sessions 3x a week for 13 wk	FBS, HbA1c, BP, lipid profile	0, 3 and 4.1 mo	Community-based participatory program is a feasible model for diabetes control. FBS decreased from 176 to 102 mg/dL ( <i>P</i> < 0.01) and HbA1c decreased from 6.9 to 6.1 ( <i>P</i> < 0.001)
Nigeria	Adeniyi <i>et al</i> <sup>[69]</sup>	To evaluate effect of 12 wk exercise program	Single group	<i>n</i> = 29, T2DM for min 6 mo, triglyceride levels > 1.7 mmol/L, waist circumference > 102 cm (men) or 88 cm (women) and BP > 130/85	Alternate day 45 min exercises (3 d in a week) for 12 wk, exercises included aerobic exercise, mobilization and resistance exercises	Fasting blood glucose, HbA1c	0, 2, 4, 6, 8, 12 wk	Improvement was observed in the fasting plasma glucose of both male ( <i>t</i> = 8.059; <i>P</i> = 0.0001) and female groups ( <i>t</i> = 13.007; <i>P</i> = 0.01)
Nigeria	Salau <i>et al</i> <sup>[57]</sup>	To evaluate effect of fruits and vegetables diet on selected hematological parameters	Single group	<i>n</i> = 30, T2DM	Two servings of diced fruit mix (100 g each) every day, 1 serving of edible green and leafy vegetables (100 g each) every day	ESR, hematocrit	0, 2, 4, 6, 8, 10 wk	ESR decreased from 49.40 to 32.8 ( <i>P</i> < 0.05). Regular intake of fruits and vegetables can reduce cardiovascular risk factors in diabetic patients

Nigeria	Oli <i>et al</i> <sup>[55]</sup>	To evaluate effect of high carbohydrate diet	Single group	<i>n</i> = 160, weight not more than 10% above or below the mean weight for their age, sex and height, age at onset of diabetes > 30 yr, random blood glucose between 100 mg/100 mL and 200 mg/100 mL, no ketonuria	250 g to 300 g of carbohydrate daily per patient depending on age and occupation	Mean fasting glucose	3 yr	Fifty-three patients (33.1%) achieved excellent control of their blood glucose (mean fasting blood glucose of 7.0 mmol/L or less); 38 patients (23.8%) achieved good control of their blood glucose (mean fasting blood glucose of 7.0-8.0 mmol/L); and 42 patients (26.3%) achieved fair control of their blood glucose (mean fasting blood, glucose of 8.0-9.0 mmol/L)
South Africa	van Rooijen <i>et al</i> <sup>[63]</sup>	To evaluate effectiveness of exercise intervention program <i>vs</i> a relaxation program	Single blind double intervention RCT	<i>n</i> = 149, 74 in relaxation group and 75 in exercise group, 40-65 yr, diagnosed T2DM for at least 1 yr	Home exercise program, fortnightly 45 min aerobics, 20 min tensing of muscles and relaxing for relaxation group, interactive group sessions, diet lectures	HbA1c, BMI, BP	0 and 3 mo	The exercise group did not impact the glycemic parameters greater than the relaxation group. HbA1c decreased -0.39 ( <i>P</i> = 0.02) for exercise group
Thailand	Komindr <i>et al</i> <sup>[58]</sup>	To evaluate effect of long-term intake of Asian food with different glycemic indices	Single group cross over	<i>n</i> = 10, T2DM, b/w 32-60 yr	High glycemic diet or low glycemic diet was mainly glutinous rice or mungbean noodles, intermediate glycemic diet was solely white rice	HbA1c	2 mo	Ingestion of mungbean noodles (a low glycemic diet) without increasing fiber intake, can improve diabetic control and protein conservation in type 2 diabetes
Thailand	Chaiopant <sup>[54]</sup>	To evaluate effect of a sitting and breathing exercise technique	Quasi-experiment, single group, pre and post-test	<i>n</i> = 50, 42-80 yr, diagnosed T2DM	Scheduled sitting and breathing techniques once a week for 30 min	Post Prandial glucose, FBS, BP	0, 1 and 2 wk	The somporn kantaradudsi-triamchaisri sitting and breathing techniques had a postprandial hypoglycemic effect in T2DM patients. Post prandial plasma glucose levels decreased from 19.26 mg/dL ( <i>P</i> < 0.001) in the 2 <sup>nd</sup> week to 17.64 mg/dL in the 3 <sup>rd</sup> week ( <i>P</i> < 0.001)
Turkey	Acik <i>et al</i> <sup>[65]</sup>	To evaluate effectiveness of education and lifestyle recommendations	Non-randomized cluster controlled trial	<i>n</i> = 80, 33 in standard diet, 28 in exercise + diet, 39 in control group, diagnosed T2DM	Nutritional counseling, structure physical activity schedule 3 times/wk	HbA1c, BMI, Blood Glucose	0, 1 and 2 mo	Diabetes education intervention program involving lifestyle modifications improves glycemic parameters. HbA1c in the diet + exercise group decreased from 9.9% to 7.9% ( <i>P</i> = 0.001) and in the diet group, levels decreased from 7.8% to 7.5% ( <i>P</i> = 0.001)
Mexico	Jimenez-Cruz <i>et al</i> <sup>[59]</sup>	To evaluate effectiveness of lower-higher-glycemic index mexican style diet	RCT crossover	<i>n</i> = 14, 35-75 yr, diagnosed T2DM	Pamphlets, detailed instructions on high-low GI foods, washout period of 6 wk with 6 wk periods of treatment alternating between low-GI period and high-GI period	FPG, HbA1c, BMI, lipid panel	0, 1.5 and 3 mo	A low-GI mexican style diet improves metabolic control in obese T2DM patients. HbA1c is lower in the low-GI period (8.1) than the high GI-period (8.6) <i>P</i> = 0.02

T2DM: Type 2 diabetes mellitus; RCT: Randomized control trial; BMI: Body mass index; GWB: General well being; PRT: Progressive resistance training; HbA1c: Hemoglobin A1c; GI: Gastrointestinal; PRT: Progressive resistance training.



found sustained glycemic control at 18 mo. Chen *et al.*<sup>[75]</sup> evaluated the efficacy of a telehealth system with diabetes education and home blood glucose monitoring in China, and found significantly lower HbA1c in the experimental group at 1 year. One study evaluated nurse SMS and follow-up *via* telephone and found significant improvements in glycemic control at 3 mo<sup>[76]</sup>. One RCT assessed efficacy of delivering SMS messages as reminders to follow diet, physical activity and prescription adherence and found significantly lower mean fasting blood glucose and 2 h post-prandial glucose in experimental group at 12 mo<sup>[77]</sup>. An RCT in Iran found a significant improvement in glycemic control in the experimental group that received electronic education (chat rooms, personal feedback from physician online)<sup>[78]</sup>. Only 1 of the 9 studies integrating technology failed to find any significant improvement in glycemic control; it was conducted in South Africa and involved a phone buddy system<sup>[79]</sup>. This study evaluated the effectiveness of a peer support mobile-phone based self-management diabetes intervention in a scarce resource setting. Women were paired with a phone buddy for support and were questioned about health behaviors *via* SMS. Blood glucose increased by 3.3 points by the end of study, but women reported higher level of social coping and they continued to attend meetings even a year later<sup>[79]</sup>.

### Diabetes prevention

Ten studies were recognized as diabetes prevention studies. These studies varied vastly in terms of sample size, study design, measurement outcomes and results. Among the 10 studies classified as prevention studies, 7 were categorized under the DPP-like interventions, as shown in Table 4. Of all the prevention studies, 4 studies were RCTs<sup>[80-84]</sup>, 3 were single group<sup>[85,86]</sup> and two group studies<sup>[87]</sup> and 1 was a non-randomized controlled trial<sup>[88]</sup>. Sample sizes ranged from a large size of  $n = 4747$  in the Iran non-randomized cluster controlled trial by Harati *et al.*<sup>[88]</sup> to a small size of  $n = 19$  in the Thailand two group experimental study by Numbenjapon *et al.*<sup>[87]</sup>. Follow-up period also varied vastly, from a minimum of 6 mo<sup>[82,86]</sup> to a maximum of 72 mo<sup>[83]</sup>. India contributed the largest number of studies with a total of 4; other study countries were China, Peru Brazil, Iran and Thailand. It is noteworthy that one of the Indian studies evaluated lifestyle education intervention in youth 15-17 years of age<sup>[82]</sup>.

Among the 7 studies that reported on interventions that were most similar to the DPP integrating lifestyle education, all reported evidence of efficacy. There was a diverse range in personnel who delivered the lifestyle education from a mix of clinicians<sup>[87]</sup> to trained nutritionists<sup>[80-82]</sup> to community health workers<sup>[86]</sup>. All studies reported significant improvements in FBG<sup>[80,85,86]</sup>, BMI<sup>[87]</sup>, HOMA-BCF<sup>[82]</sup> or cumulative incidence<sup>[81,88]</sup>. Among the 3 interventions that integrated diet and exercise as main components, all reported clinically

significant changes in outcomes, as shown in Table 5. Xu *et al.*<sup>[84]</sup> had a sample size of 81 participants and examined the effectiveness of low-glycemic meal replacement and individualized eating instructions along with exercise recommendations during a span of 12 mo. Lindgärde *et al.*<sup>[89]</sup> had a sample size of 59 participants and examined the effectiveness of structured supervised endurance training in a span of 6 mo. Both studies were RCTs and observed significant changes in fasting plasma glucose levels by the end of the study in the experimental groups<sup>[84,89]</sup>. Pan *et al.*<sup>[83]</sup> was the most ambitious trial with a sample of 530, and follow-up period of 72 mo; they evaluated whether diet alone, exercise alone, and diet and exercise combined delayed development of diabetes; they found significant reduction in diabetes incidence in all three experimental groups, when compared to control.

## DISCUSSION

There are many lessons that can be learned from this review. There is a wide range of diabetes prevention and management strategies in the developing world. We identified 66 studies in 20 different low and middle income countries; 56 out of the 66 studies reported on diabetes management, and the remainder reported on diabetes prevention trial. We aimed to (1) evaluate whether diabetes prevention and control interventions that are similar to DPP in low and middle-income country context are effective; (2) identify interventions that are substantively different from the DPP (with regard to intervention components); and (3) among this latter group, evaluate whether there is evidence of efficacy. With regard to diabetes management interventions that were similar to DPP there is overwhelming evidence to suggest that they are effective. Further, potentially lower-cost allied health professionals such as nurses and pharmacists can play central roles in delivering lifestyle and medication adherence education. Pharmacist led interventions in particular should be promoted in United States settings because of the strong evidence of efficacy and their impact on glycemic control documented in this review as well as a previous Canadian review<sup>[90]</sup>. Pharmacies are potentially more cost-effective and more accessible than other healthcare providers; they may also be able to deliver lifestyle education in addition to medication advice as seen is a Turkish and Jordan study reviewed here<sup>[46,91]</sup>. Indeed, United States insurers are recognizing the cost-savings of utilizing pharmacists for this role<sup>[92]</sup>. Further, the approach can be potentially translated to alternative ethnic-specific healthcare settings such as botanicas, for example, which are seen as an important healthcare options among United States ethnic minority populations<sup>[93]</sup> who carry a disproportionate burden of diabetes<sup>[94-97]</sup>.

Our review also highlighted the diverse approaches to structured dietary interventions in diabetes management with evidence of efficacy on improving outcomes such as HbA1c<sup>[59]</sup>. However, more research is needed in this

**Table 3** Diabetes management: Technology assisted interventions

Country	Ref.	Objective	Study design	Sample size/ characteristics	Components of intervention	Measurements	Outcome measures	Conclusion
Bangladesh	Kibriya <i>et al</i> <sup>[72]</sup>	To evaluate effectiveness of HMBG	RCT	$n = 64$ , 32 in each arm, T2DM requiring OHA/ insulin, 35-64 yr, completed secondary school education, high SES	Health Education Sessions, HMBG Practical Sessions for 2 d	FBG, HbA1c	0, 3, 6, 9, 12, 15 and 18 mo	HMBG + education is cost-effective in developing country. FBG decreased by 2.49 mmol ( $P = 0.007$ ) and HbA1c decreased by 1.37% ( $P = 0.02$ ) in experimental group. FBG decreased by 1.47 mmol ( $P = 0.051$ ) and HbA1c lost significance after 18 mo of follow up in control group
Bulgaria	Kempf <i>et al</i> <sup>[73]</sup>	To evaluate effectiveness of SMBG on T2DM patients	RCT	$n = 124$ , 63 in SMBG group, 61 in control group	Structured lifestyle guidance manual, 150 test strips with blood glucose meter	HbA1c	0, 12 wk, 18 mo	At 12 wk of intervention the SMBG group significantly improved glycated hemoglobin (HbA1c) levels [from 7.4 to 6.9 ( $P < 0.001$ )], whereas HbA1c reduction were not significant in the control group. At 1.5-yr follow-up, in the control group HbA1c increased again, reaching baseline values (7.5%). In the SMBG group HbA1c remained stable [6.9% ( $P = 0.0003$ for trend)]
China	Chen <i>et al</i> <sup>[74]</sup>	To evaluate the functionality of telehealth system	Two group experimental	$n = 64$ , 32 in experimental and 32 in control, T2DM	Telehealth device package with blood glucose meter for frequent monitoring according to recommendations, telehealth data analysis platform, telephone to contact health care professional, diabetes education	HbA1c	0 and 1 yr	HbA1c decreased from 9.5 to 8 in telehealth group ( $P < 0.001$ ), while in the control group, there was no significant improvement in HbA1c
India	Shetty <i>et al</i> <sup>[77]</sup>	To investigate feasibility of SMS	RCT	$n = 215$ , 110 in SMS group and 105 in control group, diagnosed T2DM > 5 yr, 10% < HbA1c > 7%	SMS once in 3 d as reminders to follow diet, physical activity and prescription adherence reminders	HbA1c, FBG, Lipid profile	0, 4, 8 and 12 mo	SMS communication is acceptable and it improved health outcomes for diabetic patients. Mean FPG (185 mg/ dL to 166, $P < 0.002$ ) and 2h PG 263 mg/ dL to 220, $P < 0.002$ ) levels decreased significantly in the SMS group. There was no significant difference in the mean HbA1c values in both groups
Iran	Zolfaghari <i>et al</i> <sup>[76]</sup>	To evaluate effect of nurse short SMS <i>vs</i> telephone follow-ups	RCT	$n = 80$ , 39 in SMS group and 42 in telephone follow-up group, T2DM, used oral medications only	SMS group received 6 messages every week with info on exercise, medication compliance, diet adherence; Telephone group received at least 2x a week call for 1 <sup>st</sup> month and then weekly for 2 <sup>nd</sup> and 3 <sup>rd</sup> month, each call lasting 20 min	HbA1c, BMI	0 and 3 mo	HbA1c decreased -0.93 ( $P < 0.001$ ) for telephone group and -1.01 ( $P < 0.001$ ) for SMS group. Both follow-up interventions can decrease HbA1c levels
Iran	Nesari <i>et al</i> <sup>[34]</sup>	To evaluate effect of nurse telephone follow-up	RCT	$n = 60$ , 30 in each group, < 65 yr, HbA1c > 7%	3 d diabetes self care education group before intervention, then telephone follow-up 2x/week for first month and then weekly for 2 <sup>nd</sup> and third months with 30 min duration	HbA1c	0 and 3 mo	The change in HbA1c level was significant for the experimental group after 12 wk but not for the control group (-1.87%, $P < 0.001$ for the experimental group <i>vs</i> -0.4%, $P < 0.15$ for the control group)

Iran	Moattari <i>et al</i> <sup>[78]</sup>	To evaluate effectiveness of electronic education	RCT	<i>n</i> = 48, 24 in experimental and 24 in control, diagnosed T2DM, insulin usage, ability to use website/ internet	Chat rooms, consultation service, educational films, personal file feedback from physician online	HbA1c, Lipid profile, FBG	0 and 3 mo	Electronic education program can be useful in improving metabolic parameters in T2DM patients sign differences. Change in HbA1c in experimental group was -2.03% ( <i>P</i> < 0.0001) and -0.6 in control group. FBS change was -10.87 mg/dL ( <i>P</i> = 0.681) in experimental group and -0.79 in control group
Malaysia	Ismail <i>et al</i> <sup>[74]</sup>	To evaluate effect of self-monitoring blood glucose	RCT	<i>n</i> = 105, 58 in intervention and 47 in control, T2DM, age 35-65 yr	Glucometer, health education, 2 d classes with demos of SMBG	HbA1c	0 and 6 mo	HbA1c level in the intervention group showed a statistically significant improvement of 1.3% ( <i>P</i> = 0.001; 95%CI: 0.6-2.0), relative to the control group that underwent usual care
South Africa	Rotheram-Borus <i>et al</i> <sup>[79]</sup>	To evaluate feasibility of mobile phone-based peer support intervention	Single group	<i>n</i> = 22, diagnosed T2DM	Informational support meetings, weekly success sessions	BMI, blood glucose, Coping, Hours of sleep	0, 3 and 6 mo	Although the phone buddy system resulted in positive coping styles and better sleep, glucose levels increased in participants

HMBG: Home monitoring of blood glucose; RCT: Randomized control trial; T2DM: Type 2 diabetes mellitus; MS: Short message service; SMBG: Self-monitoring of blood glucose.

**Table 4 Diabetes prevention: United States Diabetes Prevention Program like prevention interventions (allied health professionals and lay member facilitated)**

Country	Ref.	Objective	Study design	Sample Size/ characteristics	Components of intervention	Measurements	Outcome measures	Conclusion
Brazil	Pimentel <i>et al</i> <sup>[80]</sup>	To evaluate effectiveness of NEP	RCT	<i>n</i> = 67, 24 in intervention group and 43 in control group, IGT + 1 other risk factor for T2DM	Individual sessions once per month and group counseling twice per month with nutritionist	HbA1c, fasting glycemia + insulin, postprandial glycemia + insulin	0 and 12 mo	Long-term NEP improves metabolic parameters for high-risk DM individuals. Intervention group had a decrease in fasting glycemia (-14.0%, <i>P</i> = 0.03), fasting insulin (-9.0%, <i>P</i> = 0.05), postprandial glycemia (-21%, <i>P</i> = 0.02), postprandial insulin (-71.0%, <i>P</i> = 0.02) and HbA1c (-24.0%, <i>P</i> = 0.006). No significant changes were observed in control group
India	Ramachandran <i>et al</i> <sup>[81]</sup>	To determine whether lifestyle modification could influence development of diabetes in IGT individuals	RCT	<i>n</i> = 531, 136 in control, 133 in lifestyle modification, 133 in metformin, 129 in lifestyle modification and 35-55 yr, IGT	Diet advice in reduction of calories, refined carbs and fats by dietitian, exercise recommendations for at least 30 min of brisk walking each day for sedentary lifestyle, metformin initial dose 250 mg twice daily increased to 500 mg twice daily after 2 wk by physician	HbA1c, blood glucose	0, 6, 12, 18, 24, 30 and 36 mo	Lifestyle modification significantly reduced the incidence of diabetes in Asian Indians. Cumulative incidence of diabetes was 55% in 3 yr in control group, and it was significantly lower in all three intervention groups (LSM = 39.3%, MET = 40.5%, LSM + MET = 39.5%)
India	Balogopal <i>et al</i> <sup>[85]</sup>	To evaluate effectiveness of community-based lifestyle intervention on diabetes prevention	CBPR, single group	<i>n</i> = 850, 10-92 yr, village resident	Dietary advice from certified diabetes educator, stress relaxation techniques, physical activity promotion from physical education trainers, 10 one-on-one sessions with health messages	FBG, diabetes incidence, BMI, BP, nutrient composition of diet	0 and 7 mo	Educational intervention was successful in improving dietary patterns in individuals with pre-diabetes/ diabetes. FBG levels decreased from 94.4 mg/dL to 91.2 mg/dL ( <i>P</i> = 0.045)

India	Balogopal <i>et al</i> <sup>[86]</sup>	To evaluate effectiveness of community based diabetes prevention and management program	CBPR, single group	<i>n</i> = 1681, > 18 yr, village resident	Lifestyle modification, group and one-on-one counseling, 5 group sessions and 5 one-on-one encounters by community health workers	FBG, diabetes prevalence, BMI, BP, nutrient composition of diet	0 and 6 mo	Community-based participatory programs are a useful model for prevention and management of diabetes. FBG levels decreased from 96.26 mg/dL to 94.94 mg/dL ( <i>P</i> < 0.001)
India	Singhal <i>et al</i> <sup>[82]</sup>	To evaluate effectiveness of repetitive nutrition education and lifestyle intervention on adolescents in North India	RCT	<i>n</i> = 106, Intervention had 56, control had 50, 15-17 yr	Individual counseling for parents on phone every month for 10 min each, lectures of 30 min each for 10 wk, individual counseling for student every week for 1 h on diet by trained nutritionist, lifestyle and physical activity for at least 30 min, trained student volunteers for dissemination of health messages	HOMA-IR, waist circumference, HOMA-BCF, DI	0 and 6 mo	The intervention model has a potential to prevent T2DM in Indian adolescents. HOMA-BCF changed 56.7 in intervention group ( <i>P</i> = 0.003) and 24.5 in control group ( <i>P</i> = 0.002). Disposition index changed 30.3 ( <i>P</i> = 0.003) in intervention group and 8.3 in control group ( <i>P</i> = 0.01). No significant changes in fasting insulin and HOMA-IR were noted
Iran	Harati <i>et al</i> <sup>[88]</sup>	To evaluate effectiveness of lifestyle intervention in development of T2DM	Non-randomized cluster controlled trial	<i>n</i> = 4747, 2992 in control group, 1754 in lifestyle modification group, no baseline diabetes	Educational interviews and lectures, nutritional educational classes 4 d/wk, health volunteers distributed educational material	FBG, diabetes incidence, BMI, lipid profile	0 and 42 mo	Lifestyle interventions could decrease the risk of developing T2DM in the general population, not just high-risk patients. Incidence of diabetes in the control and intervention groups was 12.2 and 8.2 per 1000 person-years, respectively, with a relative risk reduction of 65% ( <i>P</i> < 0.003). FPG change from baseline was -2.9 ( <i>P</i> < 0.01)
Thailand	Numbenjapon <i>et al</i> <sup>[87]</sup>	To evaluate lifestyle modification vs combined treatment (lifestyle modification + metformin) to prevent diabetes	Two group experimental	<i>n</i> = 19, IGT, Fam Hx of T2DM	Monthly visit with nurse educator, nutritionist, physician and psychologist for 3 consecutive months, and then every 2 to 3 mo afterward	BMI, 2 h plasma glucose	1 yr	BMI and 2-h plasma level were significantly decreased after treatment in normal OGTT group ( <i>P</i> < 0.05)

NEP: Nutrition Education Program; RCT: Randomized control trial; T2DM: Type 2 diabetes mellitus; BMI: Body mass index; BCF: Beta-cell function; DI: Disposition index.

area as all the studies had small sample sizes and only one study was an RCT<sup>[59]</sup>. More effort should be made to integrate structured dietary components into diabetes management programs such as in the promotion of low-glycemic diets, a recommendation consistent with a recent 2014 review on nutritional strategies to prevent and manage diabetes<sup>[98]</sup>. Also, taking into consideration the importance of intervention translation to low-resource settings and diverse populations, Oli *et al*<sup>[55]</sup> highlights the importance of evaluating the impact of diets that consist of readily available local foods and found excellent/good blood glucose control in the presence of high

carbohydrate diets in their Nigerian sample. This finding is consistent with evidence to support that it is the quality of carbohydrates (*e.g.*, low vs high glycemic index), not quantity, which determines risk of diabetes<sup>[98,99]</sup>.

The benefits of exercise on diabetes management is well-documented<sup>[100]</sup>. However, less is known about how the different forms of exercise compare with regard to efficacy in managing diabetes. The efficacy of alternative forms of physical activity such as PRT and yoga/relaxation, in comparison to aerobic exercise or no exercise should be further evaluated and studied in United States. In 2 RCTs reported in this review, one in



**Table 5 Diabetes prevention: Structured dietary change and exercise as main components (not lifestyle education)**

Country	Ref.	Objective	Study design	Sample size/ characteristics	Components of intervention	Measurements	Outcome measures	Conclusion
China	Pan <i>et al</i> <sup>[83]</sup>	To determine whether diet and exercise interventions will delay development of NIDDM in individuals with IGT	RCT	<i>n</i> = 530, control = 133, diet = 130, exercise = 141, diet + exercise = 126, > 25 yr, IGT	Diet plans, exercise recommendations with brochures on instructions on increasing leisure physical activities and counseling sessions on daily recommended food intake, weekly for one month, monthly for three months and once every three months by physicians and nurses	FBG, 2-h fasting glucose	0, 24, 48 and 72 mo	Diet and exercise led to a significant decrease in the incidence of diabetes in individuals with IGT. The diet, exercise, and diet-plus-exercise interventions were associated with 31% ( <i>P</i> < 0.03), 46% ( <i>P</i> < 0.0005), and 42% ( <i>P</i> < 0.005) reductions in risk of developing diabetes, respectively
China	Xu <i>et al</i> <sup>[83]</sup>	Evaluate effectiveness of lifestyle intervention and meal replacement	RCT	<i>n</i> = 81, 41 in intervention, 40 in control, > 18 yr, IGR	Daily low-glycaemic meal replacement, individualized eating instructions, exercise recommendations, a dietician measured weekly intake and physician conducted medical evaluations	HbA1c, fasting plasma glucose, 1 hr plasma glucose	0 and 12 mo	HbA1c change was -0.12 ( <i>P</i> = 0.02), 2 h plasma glucose change was -1.24 ( <i>P</i> = 0.02), fasting plasma glucose change was -0.12 ( <i>P</i> = 0.001) in intervention group, no significant changes were noted in control group
Peru	Lindgärde <i>et al</i> <sup>[89]</sup>	To evaluate feasibility of supervised endurance training	RCT	<i>n</i> = 59, 33 in control group and 26 in experimental, 25-64 yr, normal plasma fasting glucose	Structured training sessions, one per week in control group and three per week for experimental group for 60 min each approved by physiotherapist	BMI, FBG, VO <sub>2</sub> max	0 and 6 mo	Supervised exercise training is a low cost safe therapy with favorable benefits. Plasma glucose levels decreased from 5.1 mmol/L to 4.1 mmol/L ( <i>P</i> < 0.001) in experimental group

RCT: Randomized control trial; IGT: Impaired glucose tolerance.

India and the other in South Africa, significant decreases in HbA1c levels in PRT or relaxation groups were found and were comparable to the decreases found in HbA1c levels in the comparison aerobic exercise groups<sup>[63,68]</sup>. This finding is consistent with previous evidence<sup>[101,102]</sup>.

Finally, the fields of public health and medicine have seen an explosion in mHealth both in the United States and abroad, particularly in low-resource settings<sup>[103-110]</sup>. mHealth broadly defined is "the use of mobile computing and communication technologies in health care and public health"<sup>[110]</sup>. This review identified several promising mHealth approaches with evidence of efficacy. Shetty *et al*<sup>[77]</sup> demonstrated efficacy of a SMS delivered reminder system to follow diet, physical activity and prescription adherence on mean fasting blood glucose in an RCT conducted in India. Similarly, in an RCT setting in Iran, Moattari *et al*<sup>[78]</sup> found significant improvement in glycemic control in the group the experimental group that received electronic education. This study created an electronic education system for patients with a personal online site with username and password where they could access their health care reports. Participants could also participate in a question/answer section where they received answers within 24 h. The health care team also sent recommendations at the end of every week to each participant *via* the online portal<sup>[78]</sup>. mHealth strategies

promise greater cost-efficiency over face-to-face interventions because settings are participant's natural environment with reduced needs for space, staff, and training. According to one review, the biggest advantages of using mobile devices, and in particular mobile phones, for health are that these devices are personal, intelligent, connected, and always with people<sup>[111]</sup>.

All diabetes prevention studies showed evidence of efficacy. It is notable that India led the world in the number the trials and further, is the only country that has tested trial in youth. Therefore, for future it is important to monitor progress in India and recognize their work as a resource for developing approaches in low and middle income countries as well a resource in developing cost-efficient approaches in more affluent countries. Among the DPP like interventions, as in diabetes management approaches, it is important to consider lower-cost allied health professionals as intervention agents delivering the lifestyle education as well as lay members of the community such as community health workers.

We should note limitations to our review. It is possible that the studies that we identified in this review do not comprise a representative sample of diabetes prevention and management efforts occurring in low and middle-income countries. Further, almost all studies selected reported favorable outcomes (63 of 66 studies) in the

management and prevention of diabetes. Therefore, there is a possibility that this review was subject to publication bias<sup>[112]</sup>. However, the objectives of our review were descriptive and qualitative in nature. We aimed to identify the range of intervention approaches to diabetes prevention and management and whether there was evidence of efficacy.

Diabetes is increasing throughout the world. There is an opportunity to test novel approaches to diabetes prevention and control using models developed in low-resource settings/countries. According to Narayan *et al.*<sup>[14]</sup>, we can apply lessons learned from the HIV/AIDS experience to the global epidemic of diabetes and non-communicable diseases in general: "Ironically, the lack of good health systems for noncommunicable diseases in many low- and middle-income countries may offer opportunities for testing innovative models in ways that cannot be done in high-income countries with mature systems".

Diabetes is increasing in the United States<sup>[113]</sup> and in countries that are the biggest contributors of immigrants to the United States such as Mexico, China, India and Philippines<sup>[114-116]</sup>. These immigrant populations often originate from countries where diabetes is also prevalent. There are 40 million immigrants in the United States, representing a twofold increase in just two decades (1990-2010), and a growth rate that is unparalleled in United States history<sup>[117]</sup>. Although Mexico has contributed the largest number of immigrants to the United States, recent data indicate that Asia has now replaced Latin America as the major region of origin for the foreign-born population in the United States. Among the newly arrived from Asia, Chinese-origin immigrants constitute the largest proportion<sup>[118]</sup>. Thus, lessons and approaches to diabetes prevention and management documented in this review are critically important to the development of approaches in the United States as well as other affluent countries where immigrants constitute a large proportion of the population.

## COMMENTS

### Background

Research on the efficacy of diabetes prevention and control efforts have been concentrated in the United States and Europe, but the burden of disease is felt around the globe. By limiting research to high-income countries the authors may neglect the potential for high- and low-income countries to learn from each other, and for leveraging global resources in the development of more cost-effective strategies. Building on the demonstrated efficacy of the United States Diabetes Prevention Program, this paper aimed to identify the newest approaches to type 2 diabetes prevention and control in the developing world context, to inform the design of approaches that can be translated to low-resource settings both in the United States and abroad.

### Research frontiers

The review (1) evaluated whether interventions similar to the United States Diabetes Prevention Program (DPP) are effective; (2) identified interventions that are substantively different from the DPP (with regard to intervention components); and (3) among this latter group, evaluated whether there is evidence of efficacy. This methodologic approach allowed us to identify strategies that had evidence of efficacy in order to inform the design of future

interventions and gaps in evidence that require further investigation.

### Innovations and breakthroughs

A total of 66 studies from 20 developing countries were gathered with publication dates through September 2014. India contributed the largest number of trials (11/66). Of the total 66 studies reviewed, all but 3 studies reported evidence of favorable outcomes in the prevention and control of type 2 diabetes. The overwhelming majority of studies reported on diabetes management (56/66), and among these more than half were structured lifestyle education programs. The evidence suggests that lifestyle education led by allied health professionals (nurses, pharmacists) were as effective as those led by physicians or a team of clinicians. The remaining diabetes management interventions focused on diet or exercise, but the evidence to recommend one approach over another was weak.

### Applications

While a wide range of approaches to diabetes exists, this review points to gaps in knowledge regarding efficacious approaches to diabetes prevention and management. It highlights the need for more large experimental studies of dietary and exercise interventions. Among these, more evidence is needed on exercise interventions comparing alternative exercise to aerobic, and on dietary interventions that compare quality of carbohydrates to quantity of carbohydrates. Also approaches using allied health professionals have promise and can potentially be more cost-effective. Finally, it is important to monitor diabetes prevention/control efforts in India as a considerable amount of research and approaches have been tested there.

### Peer-review

This manuscript presents the newest approaches to diabetes prevention and control in the developing countries. It is a manuscript of potential interest.

## REFERENCES

- 1 **Centers for Disease Control and Prevention.** National diabetes fact sheet: National estimates and general information on diabetes and prediabetes in the United States. Atlanta, GA: Centers for Disease Control and Prevention, 2011
- 2 **International Diabetes Federation.** IDF diabetes atlas. 6th ed. Available from: URL: <http://www.idf.org/diabetesatlas>
- 3 **Xu Y, Wang L, He J, Bi Y, Li M, Wang T, Wang L, Jiang Y, Dai M, Lu J, Xu M, Li Y, Hu N, Li J, Mi S, Chen CS, Li G, Mu Y, Zhao J, Kong L, Chen J, Lai S, Wang W, Zhao W, Ning G.** Prevalence and control of diabetes in Chinese adults. *JAMA* 2013; **310**: 948-959 [PMID: 24002281 DOI: 10.1001/jama.2013.168118]
- 4 **Fujimoto WY, Bergstrom RW, Boyko EJ, Kinyoun JL, Leonetti DL, Newell-Morris LL, Robinson LR, Shuman WP, Stolov WC, Tsunehara CH.** Diabetes and diabetes risk factors in second- and third-generation Japanese Americans in Seattle, Washington. *Diabetes Res Clin Pract* 1994; **24** Suppl: S43-S52 [PMID: 7859632 DOI: 10.1016/0168-8227(94)90226-7]
- 5 **Kawate R, Yamakido M, Nishimoto Y, Bennett PH, Hamman RF, Knowler WC.** Diabetes mellitus and its vascular complications in Japanese migrants on the Island of Hawaii. *Diabetes Care* 1979; **2**: 161-170 [PMID: 520120 DOI: 10.2337/diacare.2.2.161]
- 6 **Popkin BM, Gordon-Larsen P.** The nutrition transition: worldwide obesity dynamics and their determinants. *Int J Obes Relat Metab Disord* 2004; **28** Suppl 3: S2-S9 [PMID: 15543214 DOI: 10.1038/sj.ijo.0802804]
- 7 **Kelly T, Yang W, Chen CS, Reynolds K, He J.** Global burden of obesity in 2005 and projections to 2030. *Int J Obes (Lond)* 2008; **32**: 1431-1437 [PMID: 18607383 DOI: 10.1038/ijo.2008.102]
- 8 **Shaw JE, Sicree RA, Zimmet PZ.** Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract* 2010; **87**: 4-14 [PMID: 19896746 DOI: 10.1016/j.diabres.2009.10.007]
- 9 **Kutty VR, Soman CR, Joseph A, Pisharody R, Vijayakumar K.** Type 2 diabetes in southern Kerala: variation in prevalence among geographic divisions within a region. *Natl Med J India* 2000; **13**: 287-292 [PMID: 11209482]

- 10 **Misra A**, Ganda OP. Migration and its impact on adiposity and type 2 diabetes. *Nutrition* 2007; **23**: 696-708 [PMID: 17679049 DOI: 10.1016/j.nut.2007.06.008]
- 11 **Popkin BM**. The nutrition transition: an overview of world patterns of change. *Nutr Rev* 2004; **62**: S140-S143 [PMID: 15387480 DOI: 10.1111/j.1753-4887.2004.tb00084.x]
- 12 **Rivera JA**, Barquera S, González-Cossío T, Olaiz G, Sepúlveda J. Nutrition transition in Mexico and in other Latin American countries. *Nutr Rev* 2004; **62**: S149-S157 [PMID: 15387482 DOI: 10.1111/j.1753-4887.2004.tb00086.x]
- 13 **Yang W**, Lu J, Weng J, Jia W, Ji L, Xiao J, Shan Z, Liu J, Tian H, Ji Q, Zhu D, Ge J, Lin L, Chen L, Guo X, Zhao Z, Li Q, Zhou Z, Shan G, He J. Prevalence of diabetes among men and women in China. *N Engl J Med* 2010; **362**: 1090-1101 [PMID: 20335585 DOI: 10.1056/NEJMoa0908292]
- 14 **Narayan KM**, Ali MK, del Rio C, Koplan JP, Curran J. Global noncommunicable diseases—lessons from the HIV-AIDS experience. *N Engl J Med* 2011; **365**: 876-878 [PMID: 21899448 DOI: 10.1056/NEJMp1107189]
- 15 **Schwarz PE**, Lindström J, Kissimova-Scarbeck K, Szybinski Z, Barengo NC, Peltonen M, Tuomilehto J. The European perspective of type 2 diabetes prevention: diabetes in Europe—prevention using lifestyle, physical activity and nutritional intervention (DE-PLAN) project. *Exp Clin Endocrinol Diabetes* 2008; **116**: 167-172 [PMID: 18350480 DOI: 10.1055/s-2007-992115]
- 16 **Ramachandran A**, Ma RC, Snehalatha C. Diabetes in Asia. *Lancet* 2010; **375**: 408-418 [PMID: 19875164 DOI: 10.1016/S0140-6736(09)60937-5]
- 17 **Espeland MA**, Glick HA, Bertoni A, Brancati FL, Bray GA, Clark JM, Curtis JM, Egan C, Evans M, Foreyt JP, Ghazarian S, Gregg EW, Hazuda HP, Hill JO, Hire D, Horton ES, Hubbard VS, Jakicic JM, Jeffery RW, Johnson KC, Kahn SE, Kilean T, Kitabchi AE, Knowler WC, Kriska A, Lewis CE, Miller M, Montez MG, Murillo A, Nathan DM, Nyenwe E, Patricio J, Peters AL, Pi-Sunyer X, Pownall H, Redmon JB, Rushing J, Ryan DH, Safford M, Tsai AG, Wadden TA, Wing RR, Yanovski SZ, Zhang P. Impact of an intensive lifestyle intervention on use and cost of medical services among overweight and obese adults with type 2 diabetes: the action for health in diabetes. *Diabetes Care* 2014; **37**: 2548-2556 [PMID: 25147253 DOI: 10.2337/dc14-0093]
- 18 **American Diabetes Association**. Economic costs of diabetes in the U.S. in 2012. *Diabetes Care* 2013; **36**: 1033-1046 [PMID: 23468086 DOI: 10.2337/dc12-2625]
- 19 **Thorpe KE**, Ogden LL, Galaktionova K. Chronic conditions account for rise in Medicare spending from 1987 to 2006. *Health Aff (Millwood)* 2010; **29**: 718-724 [PMID: 20167626 DOI: 10.1377/hlthaff.2009.0474]
- 20 **Ali MK**, Echouffo-Tcheugui J, Williamson DF. How effective were lifestyle interventions in real-world settings that were modeled on the Diabetes Prevention Program? *Health Aff (Millwood)* 2012; **31**: 67-75 [PMID: 22232096]
- 21 **Johnson M**, Jones R, Freeman C, Woods HB, Gillett M, Goyder E, Payne N. Can diabetes prevention programmes be translated effectively into real-world settings and still deliver improved outcomes? A synthesis of evidence. *Diabet Med* 2013; **30**: 3-15 [PMID: 22998334 DOI: 10.1111/dme.12018]
- 22 **Knowler WC**, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 2002; **346**: 393-403 [PMID: 11832527 DOI: 10.1056/NEJMoa012512]
- 23 **The World Bank**. New Country Classifications. Available from: URL: <http://data.worldbank.org/news/new-country-classifications>
- 24 **Glasgow RE**. Evaluation of theory-based interventions: The RE-AIM Model, in health behavior and health education. In: Glanz K, Rimer BK, Lewis FM, editors. San Francisco, CA: Jossey-Bass, 2002: 530-544
- 25 **Mollaoglu M**, Beyazit E. Influence of diabetic education on patient metabolic control. *Appl Nurs Res* 2009; **22**: 183-190 [PMID: 19616166 DOI: 10.1016/j.apnr.2007.12.003]
- 26 **Tankova T**, Dakovska G, Koev D. Education and quality of life in diabetic patients. *Patient Educ Couns* 2004; **53**: 285-290 [PMID: 15186865 DOI: 10.1016/j.pec.2003.09.013]
- 27 **Sarrafzadegan N**, Kelishadi R, Sadri G, Malekafzali H, Pourmoghaddas M, Heidari K, Shirani S, Bahonar A, Boshtam M, Asgary S, Mohammadifard N, Sadeghi M, Eshtrati B, Hadipour E, Esmailzadeh A, O'Loughlin JL. Outcomes of a comprehensive healthy lifestyle program on cardiometabolic risk factors in a developing country: the Isfahan Healthy Heart Program. *Arch Iran Med* 2013; **16**: 4-11 [PMID: 23273227]
- 28 **Liu S**, Bi A, Fu D, Fu H, Luo W, Ma X, Zhuang L. Effectiveness of using group visit model to support diabetes patient self-management in rural communities of Shanghai: a randomized controlled trial. *BMC Public Health* 2012; **12**: 1043 [PMID: 23198694 DOI: 10.1186/1471-2458-12-1043]
- 29 **Gagliardino JJ**, Lapertosa S, Pflirter G, Villagra M, Caporale JE, Gonzalez CD, Elgart J, Gonzalez L, Cernadas C, Rucci E, Clark C. Clinical, metabolic and psychological outcomes and treatment costs of a prospective randomized trial based on different educational strategies to improve diabetes care (PRODIACOR). *Diabet Med* 2013; **30**: 1102-1111 [PMID: 23668772 DOI: 10.1111/dme.12230]
- 30 **Cezaretto A**, Siqueira-Catania A, de Barros CR, Salvador EP, Ferreira SR. Benefits on quality of life concomitant to metabolic improvement in intervention program for prevention of diabetes mellitus. *Qual Life Res* 2012; **21**: 105-113 [PMID: 21538199 DOI: 10.1007/s11136-011-9919-2]
- 31 **Chaves-Fonseca RM**, Matos OS, Lordelo RA, Abreu M, Farias MG, Coutinho JF, Ribeiro MN, Matteoni-Athayde L, Lessa I, Pousada J, Oliveira M, Lopes C, Strock E, Mazze R. Implementation of a systematic approach to diabetes in primary care in Bahia, Brazil improves metabolic outcomes: PRODIBA-Programa de Interiorização da Assistência ao Diabetes na Bahia (Project for Dissemination of Diabetes Care in the State of Bahia). *Diabet Med* 2009; **26**: 286-292 [PMID: 19317824 DOI: 10.1111/j.1464-5491.2008.02656.x]
- 32 **Jenhani M**, Gaha K, Nabouli R, Ghedira A, Ben Abdelaziz A. Effectiveness of patient education on glycemic control in insulin treated patients in general practice. *Diabetes Metab* 2005; **31**: 376-381 [PMID: 16369200]
- 33 **Tan MY**, Magarey JM, Chee SS, Lee LF, Tan MH. A brief structured education programme enhances self-care practices and improves glycaemic control in Malaysians with poorly controlled diabetes. *Health Educ Res* 2011; **26**: 896-907 [PMID: 21715653 DOI: 10.1093/her/cyr047]
- 34 **Nesari M**, Zakerimoghdam M, Rajab A, Bassampour S, Faghihzadeh S. Effect of telephone follow-up on adherence to a diabetes therapeutic regimen. *Jpn J Nurs Sci* 2010; **7**: 121-128 [PMID: 21092015 DOI: 10.1111/j.1742-7924.2010.00146.x]
- 35 **Kengne AP**, Fezeu L, Sobngwi E, Awah PK, Aspray TJ, Unwin NC, Mbanya JC. Type 2 diabetes management in nurse-led primary healthcare settings in urban and rural Cameroon. *Prim Care Diabetes* 2009; **3**: 181-188 [PMID: 19748331 DOI: 10.1016/j.pcd.2009.08.005]
- 36 **Labhardt ND**, Balo JR, Ndam M, Grimm JJ, Manga E. Task shifting to non-physician clinicians for integrated management of hypertension and diabetes in rural Cameroon: a programme assessment at two years. *BMC Health Serv Res* 2010; **10**: 339 [PMID: 21144064 DOI: 10.1186/1472-6963-10-339]
- 37 **DePue JD**, Rosen RK, Seiden A, Bereolos N, Chima ML, Goldstein MG, Nu'usolia O, Tuitele J, McGarvey ST. Implementation of a culturally tailored diabetes intervention with community health workers in American Samoa. *Diabetes Educ* 2013; **39**: 761-771 [PMID: 24052204 DOI: 10.1177/0145721713504630]
- 38 **Kitiş Y**, Emiroğlu ON. The effects of home monitoring by public health nurse on individuals' diabetes control. *Appl Nurs Res* 2006; **19**: 134-143 [PMID: 16877192 DOI: 10.1016/j.apnr.2005.07.007]
- 39 **Gallegos EC**, Ovalle-Berumen F, Gomez-Meza MV. Metabolic control of adults with type 2 diabetes mellitus through education and counseling. *J Nurs Scholarsh* 2006; **38**: 344-351 [PMID: 17181082]
- 40 **Price C**, Shandu D, Dedicoat M, Wilkinson D, Gill GV. Long-term glycaemic outcome of structured nurse-led diabetes care in rural Africa. *QJM* 2011; **104**: 571-574 [PMID: 21278061 DOI: 10.1093/qjmed/hcr005]



- 41 **Gill GV**, Price C, Shandu D, Dedicoat M, Wilkinson D. An effective system of nurse-led diabetes care in rural Africa. *Diabet Med* 2008; **25**: 606-611 [PMID: 18445175 DOI: 10.1111/j.1464-5491.2008.02421.x]
- 42 **Wattana C**, Srisuphan W, Pothiban L, Upchurch SL. Effects of a diabetes self-management program on glycemic control, coronary heart disease risk, and quality of life among Thai patients with type 2 diabetes. *Nurs Health Sci* 2007; **9**: 135-141 [PMID: 17470188 DOI: 10.1111/j.1442-2018.2007.00315.x]
- 43 **Navicharern R**, Aungsueroch Y, Thanasilp S. Effects of multifaceted nurse-coaching intervention on diabetic complications and satisfaction of persons with type 2 diabetes. *J Med Assoc Thai* 2009; **92**: 1102-1112 [PMID: 19694337]
- 44 **Oba N**, McCaffrey R, Choonhapran P, Chutug P, Rueangram S. Development of a community participation program for diabetes mellitus prevention in a primary care unit, Thailand. *Nurs Health Sci* 2011; **13**: 352-359 [PMID: 21812881 DOI: 10.1111/j.1442-2018.2011.00627.x]
- 45 **Suppaitiporn S**, Chindavijak B, Onsanit S. Effect of diabetes drug counseling by pharmacist, diabetic disease booklet and special medication containers on glycemic control of type 2 diabetes mellitus: a randomized controlled trial. *J Med Assoc Thai* 2005; **88** Suppl 4: S134-S141 [PMID: 16623018]
- 46 **Turnacilar M**, Sancar M, Apikoglu-Rabus S, Hursitoglu M, Izzettin FV. Improvement of diabetes indices of care by a short pharmaceutical care program. *Pharm World Sci* 2009; **31**: 689-695 [PMID: 19777365 DOI: 10.1007/s11096-009-9333-9]
- 47 **Farsaei S**, Sabzghabae AM, Zargarzadeh AH, Amini M. Effect of pharmacist-led patient education on glycemic control of type 2 diabetes: a randomized controlled trial. *J Res Med Sci* 2011; **16**: 43-49 [PMID: 21448382]
- 48 **Jarab AS**, Alqudah SG, Mukattash TL, Shattat G, Al-Qirim T. Randomized controlled trial of clinical pharmacy management of patients with type 2 diabetes in an outpatient diabetes clinic in Jordan. *J Manag Care Pharm* 2012; **18**: 516-526 [PMID: 22971205]
- 49 **Mourão AO**, Ferreira WR, Martins MA, Reis AM, Carrillo MR, Guimarães AG, Ev LS. Pharmaceutical care program for type 2 diabetes patients in Brazil: a randomised controlled trial. *Int J Clin Pharm* 2013; **35**: 79-86 [PMID: 23161124 DOI: 10.1007/s11096-012-9710-7]
- 50 **Correr CJ**, Melchior AC, Fernandez-Llamos F, Pontarolo R. Effects of a pharmacotherapy follow-up in community pharmacies on type 2 diabetes patients in Brazil. *Int J Clin Pharm* 2011; **33**: 273-280 [PMID: 21394570 DOI: 10.1007/s11096-011-9493-2]
- 51 **Borges AP**, Guidoni CM, Ferreira LD, de Freitas O, Pereira LR. The pharmaceutical care of patients with type 2 diabetes mellitus. *Pharm World Sci* 2010; **32**: 730-736 [PMID: 20734138 DOI: 10.1007/s11096-010-9428-3]
- 52 **Petkova VB**, Petrova GI. Pilot project for education of patients with type 2 diabetes by pharmacists. *Acta Diabetol* 2006; **43**: 37-42 [PMID: 16865327 DOI: 10.1007/s00592-006-0209-3]
- 53 **Less LA**, Ragoobirsingh D, Morrison EY, Boyne M, Johnson PA. A preliminary report on an assessment of a community-based intervention for diabetes control in adults with type 2 diabetes. *Fam Pract* 2010; **27** Suppl 1: i46-i52 [PMID: 19965903 DOI: 10.1093/fampra/cmp085]
- 54 **Chaiopanont S**. Hypoglycemic effect of sitting breathing meditation exercise on type 2 diabetes at Wat Khae Nok Primary Health Center in Nonthaburi province. *J Med Assoc Thai* 2008; **91**: 93-98 [PMID: 18386551]
- 55 **Oli JM**, Ikeakor IP. High carbohydrate diet in the management of non-obese non-insulin-dependent Nigerian diabetics. *Hum Nutr Appl Nutr* 1984; **38**: 479-486 [PMID: 6526692]
- 56 **Pande A**, Krishnamoorthy G, Moulick ND. Hypoglycaemic and hypolipidaemic effects of low GI and medium GL Indian diets in type 2 diabetes for a period of 4 weeks: a prospective study. *Int J Food Sci Nutr* 2012; **63**: 649-658 [PMID: 22229934 DOI: 10.3109/09637486.2011.649247]
- 57 **Salau BA**, Adeyanju MM, Odufuwa KT, Osilesi O. Fruits and vegetables diet improves some selected haemorrhological parameters predisposing to cardiovascular disease in non insulin dependent diabetes mellitus NIDDM subjects. *Pak J Biol Sci* 2012; **15**: 694-697 [PMID: 24171252 DOI: 10.3923/pjbs.2012.694.697]
- 58 **Komindr S**, Ingsriswang S, Lerdvuthisopon N, Boontawee A. Effect of long-term intake of Asian food with different glycemic indices on diabetic control and protein conservation in type 2 diabetic patients. *J Med Assoc Thai* 2001; **84**: 85-97 [PMID: 11281505]
- 59 **Jimenez-Cruz A**, Bacardi-Gascon M, Turnbull WH, Rosales-Garay P, Severino-Lugo I. A flexible, low-glycemic index mexican-style diet in overweight and obese subjects with type 2 diabetes improves metabolic parameters during a 6-week treatment period. *Diabetes Care* 2003; **26**: 1967-1970 [PMID: 12832297 DOI: 10.2337/diacare.26.7.1967]
- 60 **Rodrigues Silva C**, Dutra de Oliveira JE, de Souza RA, Silva HC. Effect of a rice bran fiber diet on serum glucose levels of diabetic patients in Brazil. *Arch Latinoam Nutr* 2005; **55**: 23-27 [PMID: 16187674]
- 61 **Agrawal RP**, Sharma P, Gafoorunnisa SJ, Ibrahim SA, Shah B, Shukla DK, Kaur T. Effect of camel milk on glucose metabolism in adults with normal glucose tolerance and type 2 diabetes in Raica community: a crossover study. *Acta Biomed* 2011; **82**: 181-186 [PMID: 22783713]
- 62 **Shenoy S**, Guglani R, Sandhu JS. Effectiveness of an aerobic walking program using heart rate monitor and pedometer on the parameters of diabetes control in Asian Indians with type 2 diabetes. *Prim Care Diabetes* 2010; **4**: 41-45 [PMID: 19945929 DOI: 10.1016/j.pcd.2009.10.004]
- 63 **van Rooijen AJ**, Rheeder P, Eales CJ, Becker PJ. Effect of exercise versus relaxation on haemoglobin A1C in Black females with type 2 diabetes mellitus. *QJM* 2004; **97**: 343-351 [PMID: 15152108 DOI: 10.1093/qjmed/hch061]
- 64 **Goldhaber-Fiebert JD**, Goldhaber-Fiebert SN, Tristán ML, Nathan DM. Randomized controlled community-based nutrition and exercise intervention improves glycemia and cardiovascular risk factors in type 2 diabetic patients in rural Costa Rica. *Diabetes Care* 2003; **26**: 24-29 [PMID: 12502654 DOI: 10.2337/diacare.26.1.24]
- 65 **Acik Y**, Bulut HY, Gulbayrak C, Ardicoglu O, Ilhan N. Effectiveness of a diabetes education and intervention program on blood glucose control for patients with type 2 diabetes in a Turkish community. *Southeast Asian J Trop Med Public Health* 2004; **35**: 1012-1018 [PMID: 15916107]
- 66 **Yazdanpanah B**, Safari M, Yazdanpanah Sh, Angha P, Karami M, Emadi M, Yazdanpanah S, Poorbehesht A. The effect of participatory community-based diabetes cares on the control of diabetes and its risk factors in western suburb of Yasouj, Iran. *Health Educ Res* 2012; **27**: 794-803 [PMID: 22907534 DOI: 10.1093/her/cys079]
- 67 **Misra A**, Alappan NK, Vikram NK, Goel K, Gupta N, Mittal K, Bhatt S, Luthra K. Effect of supervised progressive resistance-exercise training protocol on insulin sensitivity, glycemia, lipids, and body composition in Asian Indians with type 2 diabetes. *Diabetes Care* 2008; **31**: 1282-1287 [PMID: 18316394 DOI: 10.2337/dc07-2316]
- 68 **Arora E**, Shenoy S, Sandhu JS. Effects of resistance training on metabolic profile of adults with type 2 diabetes. *Indian J Med Res* 2009; **129**: 515-519 [PMID: 19675378]
- 69 **Adeniyi AF**, Uloko AE, Ogwumike OO, Sanya AO, Fasanmade AA. Time course of improvement of metabolic parameters after a 12 week physical exercise programme in patients with type 2 diabetes: the influence of gender in a Nigerian population. *Biomed Res Int* 2013; **2013**: 310574 [PMID: 24078913 DOI: 10.1155/2013/310574]
- 70 **Kosuri M**, Sridhar GR. Yoga practice in diabetes improves physical and psychological outcomes. *Metab Syndr Relat Disord* 2009; **7**: 515-517 [PMID: 19900155 DOI: 10.1089/met.2009.0011]
- 71 **Sun J**, Wang Y, Chen X, Chen Y, Feng Y, Zhang X, Pan Y, Hu T, Xu J, Du L, Zhou W, Zhao H, Riley RE, Mustad VA. An integrated intervention program to control diabetes in overweight Chinese women and men with type 2 diabetes. *Asia Pac J Clin Nutr* 2008; **17**: 514-524 [PMID: 18818173]
- 72 **Kibriya MG**, Ali L, Banik NG, Khan AK. Home monitoring of blood glucose (HMBG) in Type-2 diabetes mellitus in a developing country. *Diabetes Res Clin Pract* 1999; **46**: 253-257 [PMID: 10624792]



- 73 **Kempf K**, Tankova T, Martin S. ROSSO-in-praxi-international: long-term effects of self-monitoring of blood glucose on glucometabolic control in patients with type 2 diabetes mellitus not treated with insulin. *Diabetes Technol Ther* 2013; **15**: 89-96 [PMID: 23194054 DOI: 10.1089/dia.2012.0213]
- 74 **Ismail M**, Teng CL, Omar M, Ho BK, Kusiar Z, Hasim R. Usage of glucometer is associated with improved glycaemic control in type 2 diabetes mellitus patients in Malaysian public primary care clinics: an open-label, randomised controlled trial. *Singapore Med J* 2013; **54**: 391-395 [PMID: 23900469]
- 75 **Chen SY**, Chang YH, Hsu HC, Lee YJ, Hung YJ, Hsieh CH. One-year efficacy and safety of the telehealth system in poorly controlled type 2 diabetic patients receiving insulin therapy. *Telemed J E Health* 2011; **17**: 683-687 [PMID: 21882998 DOI: 10.1089/tmj.2011.0020]
- 76 **Zolfaghari M**, Mousavifar SA, Pedram S, Haghani H. The impact of nurse short message services and telephone follow-ups on diabetic adherence: which one is more effective? *J Clin Nurs* 2012; **21**: 1922-1931 [PMID: 22239205 DOI: 10.1111/j.1365-2702.2011.03951.x]
- 77 **Shetty AS**, Chamukuttan S, Nanditha A, Raj RK, Ramachandran A. Reinforcement of adherence to prescription recommendations in Asian Indian diabetes patients using short message service (SMS)-a pilot study. *J Assoc Physicians India* 2011; **59**: 711-714 [PMID: 22616337]
- 78 **Moattari M**, Hashemi M, Dabbaghmanesh MH. The impact of electronic education on metabolic control indicators in patients with diabetes who need insulin: a randomised clinical control trial. *J Clin Nurs* 2013; **22**: 32-38 [PMID: 22905971 DOI: 10.1111/j.1365-2702.2012.04200.x]
- 79 **Rotheram-Borus MJ**, Tomlinson M, Gwegwe M, Comulada WS, Kaufman N, Keim M. Diabetes buddies: peer support through a mobile phone buddy system. *Diabetes Educ* 2012; **38**: 357-365 [PMID: 22546740 DOI: 10.1177/0145721712444617]
- 80 **Pimentel GD**, Portero-McLellan KC, Oliveira EP, Spada AP, Oshiiwa M, Zemdeg JC, Barbalho SM. Long-term nutrition education reduces several risk factors for type 2 diabetes mellitus in Brazilians with impaired glucose tolerance. *Nutr Res* 2010; **30**: 186-190 [PMID: 20417879 DOI: 10.1016/j.nutres.2010.03.003]
- 81 **Ramachandran A**, Snehalatha C, Mary S, Mukesh B, Bhaskar AD, Vijay V. The Indian Diabetes Prevention Programme shows that lifestyle modification and metformin prevent type 2 diabetes in Asian Indian subjects with impaired glucose tolerance (IDPP-1). *Diabetologia* 2006; **49**: 289-297 [PMID: 16391903 DOI: 10.1007/s00125-005-0097-z]
- 82 **Singhal N**, Misra A, Shah P, Gulati S, Bhatt S, Sharma S, Pandey RM. Impact of intensive school-based nutrition education and lifestyle interventions on insulin resistance,  $\beta$ -cell function, disposition index, and subclinical inflammation among Asian Indian adolescents: a controlled intervention study. *Metab Syndr Relat Disord* 2011; **9**: 143-150 [PMID: 21118028 DOI: 10.1089/met.2010.0094]
- 83 **Pan XR**, Li GW, Hu YH, Wang JX, Yang WY, An ZX, Hu ZX, Lin J, Xiao JZ, Cao HB, Liu PA, Jiang XG, Jiang YY, Wang JP, Zheng H, Zhang H, Bennett PH, Howard BV. Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance. The Da Qing IGT and Diabetes Study. *Diabetes Care* 1997; **20**: 537-544 [PMID: 9096977]
- 84 **Xu DF**, Sun JQ, Chen M, Chen YQ, Xie H, Sun WJ, Lin YF, Jiang JJ, Sun W, Chen AF, Tang QR. Effects of lifestyle intervention and meal replacement on glycaemic and body-weight control in Chinese subjects with impaired glucose regulation: a 1-year randomised controlled trial. *Br J Nutr* 2013; **109**: 487-492 [PMID: 23021205 DOI: 10.1017/S0007114512001328]
- 85 **Balagopal P**, Kamalamma N, Patel TG, Misra R. A community-based diabetes prevention and management education program in a rural village in India. *Diabetes Care* 2008; **31**: 1097-1104 [PMID: 18316397 DOI: 10.2337/dc07-1680]
- 86 **Balagopal P**, Kamalamma N, Patel TG, Misra R. A community-based participatory diabetes prevention and management intervention in rural India using community health workers. *Diabetes Educ* 2012; **38**: 822-834 [PMID: 23033123 DOI: 10.1177/0145721712459890]
- 87 **Numbenjapon N**, Nakavachara P, Santiprabhob J, Kiattisakthavee P, Wongam R, Likitmaskul S. Successful strategy to improve glucose tolerance in Thai obese youth. *J Med Assoc Thai* 2010; **93** Suppl 6: S131-S138 [PMID: 21280526]
- 88 **Harati H**, Hadaegh F, Momenan AA, Ghanei L, Bozorgmanesh MR, Ghanbarian A, Mirmiran P, Azizi F. Reduction in incidence of type 2 diabetes by lifestyle intervention in a middle eastern community. *Am J Prev Med* 2010; **38**: 628-636.e1 [PMID: 20494239 DOI: 10.1016/j.amepre.2010.03.003]
- 89 **Lindgärde F**, Åhrén B. Improved metabolic risk markers following two 6-month physical activity programs among socioeconomic marginalized women of Native American ancestry in Lima, Peru. *Diabetes Care* 2007; **30**: 2230-2232 [PMID: 17540957 DOI: 10.2337/dc06-2633]
- 90 **Machado M**, Bajcar J, Guzzo GC, Einarson TR. Sensitivity of patient outcomes to pharmacist interventions. Part II: Systematic review and meta-analysis in hypertension management. *Ann Pharmacother* 2007; **41**: 1770-1781 [PMID: 17925496 DOI: 10.1345/aph.1K311]
- 91 **Jarab AS**, Alqudah SG, Khdour M, Shamsain M, Mukattash TL. Impact of pharmaceutical care on health outcomes in patients with COPD. *Int J Clin Pharm* 2012; **34**: 53-62 [PMID: 22101426 DOI: 10.1007/s11096-011-9585-z]
- 92 **Abelson R**. An Insurer's New Approach to Diabetes. Available from: URL: [http://www.nytimes.com/2010/04/14/health/14diabetes.html?pagewanted=all&\\_r=0](http://www.nytimes.com/2010/04/14/health/14diabetes.html?pagewanted=all&_r=0)
- 93 **Gomez-Beloz A**, Chavez N. The botánica as a culturally appropriate health care option for Latinos. *J Altern Complement Med* 2001; **7**: 537-546 [PMID: 11719946 DOI: 10.1089/10755530152639765]
- 94 **Gardner LI**, Stern MP, Haffner SM, Gaskill SP, Hazuda HP, Relethford JH, Eifler CW. Prevalence of diabetes in Mexican Americans. Relationship to percent of gene pool derived from native American sources. *Diabetes* 1984; **33**: 86-92 [PMID: 6690348 DOI: 10.2337/diab.33.1.86]
- 95 **Stern MP**, Knapp JA, Hazuda HP, Haffner SM, Patterson JK, Mitchell BD. Genetic and environmental determinants of type II diabetes in Mexican Americans. Is there a "descending limb" to the modernization/diabetes relationship? *Diabetes Care* 1991; **14**: 649-654 [PMID: 1914814 DOI: 10.2337/diacare.14.7.649]
- 96 **Elbein SC**. Genetics factors contributing to type 2 diabetes across ethnicities. *J Diabetes Sci Technol* 2009; **3**: 685-689 [PMID: 20144314 DOI: 10.1177/193229680900300412]
- 97 **Haffner SM**. Epidemiology of type 2 diabetes: risk factors. *Diabetes Care* 1998; **21** Suppl 3: C3-C6 [PMID: 9850478 DOI: 10.2337/diacare.21.3.C3]
- 98 **Ley SH**, Hamdy O, Mohan V, Hu FB. Prevention and management of type 2 diabetes: dietary components and nutritional strategies. *Lancet* 2014; **383**: 1999-2007 [PMID: 24910231 DOI: 10.1016/S0140-6736(14)60613-9]
- 99 **Bhupathiraju SN**, Tobias DK, Malik VS, Pan A, Hruby A, Manson JE, Willett WC, Hu FB. Glycemic index, glycemic load, and risk of type 2 diabetes: results from 3 large US cohorts and an updated meta-analysis. *Am J Clin Nutr* 2014; **100**: 218-232 [PMID: 24787496 DOI: 10.3945/ajcn.113.079533]
- 100 **Thomas DE**, Elliott EJ, Naughton GA. Exercise for type 2 diabetes mellitus. *Cochrane Database Syst Rev* 2006; **(3)**: CD002968 [PMID: 16855995 DOI: 10.1002/14651858.CD002968.pub2]
- 101 **Ng CL**, Goh SY, Malhotra R, Østbye T, Tai ES. Minimal difference between aerobic and progressive resistance exercise on metabolic profile and fitness in older adults with diabetes mellitus: a randomised trial. *J Physiother* 2010; **56**: 163-170 [PMID: 20795922]
- 102 **Irvine C**, Taylor NF. Progressive resistance exercise improves glycaemic control in people with type 2 diabetes mellitus: a systematic review. *Aust J Physiother* 2009; **55**: 237-246 [PMID: 19929766]
- 103 **Chávez NR**, Shearer LS, Rosenthal SL. Use of digital media technology for primary prevention of STIs/HIV in youth. *J Pediatr Adolesc Gynecol* 2014; **27**: 244-257 [PMID: 24332613 DOI: 10.1016/j.jpag.2013.07.008]
- 104 **Finitis DJ**, Pellowski JA, Johnson BT. Text message intervention designs to promote adherence to antiretroviral therapy (ART): a meta-

- analysis of randomized controlled trials. *PLoS One* 2014; **9**: e88166 [PMID: 24505411 DOI: 10.1371/journal.pone.0088166]
- 105 **Horvath T**, Azman H, Kennedy GE, Rutherford GW. Mobile phone text messaging for promoting adherence to antiretroviral therapy in patients with HIV infection. *Cochrane Database Syst Rev* 2012; **3**: CD009756 [PMID: 22419345 DOI: 10.1002/14651858.CD009756]
  - 106 **Vodopivec-Jamsek V**, de Jongh T, Gurol-Urganci I, Atun R, Car J. Mobile phone messaging for preventive health care. *Cochrane Database Syst Rev* 2012; **12**: CD007457 [PMID: 23235643 DOI: 10.1002/14651858.CD007457.pub2]
  - 107 **Free C**, Phillips G, Watson L, Galli L, Felix L, Edwards P, Patel V, Haines A. The effectiveness of mobile-health technologies to improve health care service delivery processes: a systematic review and meta-analysis. *PLoS Med* 2013; **10**: e1001363 [PMID: 23458994 DOI: 10.1371/journal.pmed.1001363]
  - 108 **Shiffman S**. Ecological momentary assessment (EMA) in studies of substance use. *Psychol Assess* 2009; **21**: 486-497 [PMID: 19947783 DOI: 10.1037/a0017074]
  - 109 **Aranda-Jan CB**, Mohutsiwa-Dibe N, Loukanova S. Systematic review on what works, what does not work and why of implementation of mobile health (mHealth) projects in Africa. *BMC Public Health* 2014; **14**: 188 [PMID: 24555733 DOI: 10.1186/1471-2458-14-188]
  - 110 **Free C**, Phillips G, Felix L, Galli L, Patel V, Edwards P. The effectiveness of M-health technologies for improving health and health services: a systematic review protocol. *BMC Res Notes* 2010; **3**: 250 [PMID: 20925916 DOI: 10.1186/1756-0500-3-250]
  - 111 **Fiordelli M**, Diviani N, Schulz PJ. Mapping mHealth research: a decade of evolution. *J Med Internet Res* 2013; **15**: e95 [PMID: 23697600 DOI: 10.2196/jmir.2430]
  - 112 **Haidich AB**. Meta-analysis in medical research. *Hippokratia* 2010; **14**: 29-37 [PMID: 21487488]
  - 113 **Centers for Disease Control and Prevention**. National Center for Health Statistics, Division of Health Interview Statistics, data from the National Health Interview Survey. Statistical analysis by the Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Division of Diabetes Translation. Available from: URL: <http://www.cdc.gov/diabetes/statistics/prev/national/figage.htm>
  - 114 **Villalpando S**, de la Cruz V, Rojas R, Shamah-Levy T, Avila MA, Gaona B, Rebollar R, Hernández L. Prevalence and distribution of type 2 diabetes mellitus in Mexican adult population: a probabilistic survey. *Salud Publica Mex* 2010; **52** Suppl 1: S19-S26 [PMID: 20585724]
  - 115 **Yang SH**, Dou KF, Song WJ. Prevalence of diabetes among men and women in China. *N Engl J Med* 2010; **362**: 2425-2426; author reply 2426 [PMID: 20578276]
  - 116 **Soria ML**, Sy RG, Vega BS, Ty-Willing T, Abenir-Gallardo A, Velandria F, Punzalan FE. The incidence of type 2 diabetes mellitus in the Philippines: a 9-year cohort study. *Diabetes Res Clin Pract* 2009; **86**: 130-133 [PMID: 19766344 DOI: 10.1016/j.diabetes.2009.07.014]
  - 117 **Malone N**, Baluja KF, Costanzo JM, Davis CJ. The Foreign-Born Population: 2000. Census 2000 Brief. United States: Census Bureau, 2003
  - 118 **Walters NP**, Trevelyan EN. The newly arrived foreign-born population of the United States: 2010. American Community Survey Briefs. United States: Census Bureau, 2011
  - 119 **Chan MF**, Yee AS, Leung EL, Day MC. The effectiveness of a diabetes nurse clinic in treating older patients with type 2 diabetes for their glycaemic control. *J Clin Nurs* 2006; **15**: 770-781 [PMID: 16684173 DOI: 10.1111/j.1365-2702.2006.01357.x]

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*World Journal of Diabetes*

Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China  
Telephone: +86-10-85381891

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Clinical Trials Study

## Prediction of the effect on antihyperglycaemic action of sitagliptin by plasma active form glucagon-like peptide-1

Akifumi Kushiya, Takako Kikuchi, Kentaro Tanaka, Tazu Tahara, Toshiko Takao, Yukiko Onishi, Yoko Yoshida, Shoji Kawazu, Yasuhiko Iwamoto

Akifumi Kushiya, Takako Kikuchi, Kentaro Tanaka, Tazu Tahara, Toshiko Takao, Yukiko Onishi, Yoko Yoshida, Shoji Kawazu, Yasuhiko Iwamoto, Division of Diabetes and Metabolism, Institute for Adult Diseases, Asahi Life Foundation, Tokyo 103-0002, Japan

Kentaro Tanaka, Department of Nephrology, School of Medicine, Faculty of Medicine, Toho University, Tokyo 103-0002, Japan

**Author contributions:** Kushiya A designed research; Kikuchi T, Tahara T, Takao T, Onishi Y and Yoshida Y performed research; Kushiya A and Tanaka K analyzed data; Kushiya A, Kawazu S and Iwamoto Y wrote paper.

**Institutional review board statement:** The protocol was approved by the Institutional Review Board (IRB) of the Institute for Adult Diseases, Asahi Life Foundation.

**Clinical trial registration statement:** The study is a prospective, single-arm study and was registered at UMIN-CTR (Registration NO: UMIN000010645).

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**Correspondence to:** Akifumi Kushiya, MD, PhD, Division of Diabetes and Metabolism, Institute for Adult Diseases, Asahi Life Foundation, 2-2-6 Nihonbashi, Bakurocho, Chuo-ku, Tokyo

103-0002, Japan. [kusiya-tky@umin.ac.jp](mailto:kusiya-tky@umin.ac.jp)  
 Telephone: +81-3-3639-5501  
 Fax: +81-3-36395520

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### Abstract

**AIM:** To investigate whether active glucagon-like peptide-1 (GLP-1) is a prediction Factor of Effect of sitagliptin on patients with type 2 diabetes mellitus (GLP-1 FST: UMIN000010645).

**METHODS:** Seventy-six patients with type 2 diabetes, who had insufficient glycemic control [Hemoglobin A1c (HbA1c)  $\geq 7\%$ ] in spite of treatment with metformin and/or sulfonylurea, were included in the investigation. Patients were divided into three groups by tertiles of fasting plasma active GLP-1 level, before the administration of 50 mg sitagliptin.

**RESULTS:** At baseline, body mass index, serum UA, insulin and HOMA-IR were higher in the high active GLP-1 group than in the other two groups. The high active GLP-1 group did not show any decline of HbA1c ( $7.6\% \pm 1.4\%$  to  $7.5\% \pm 1.5\%$ ), whereas the middle and low groups indicated significant decline of HbA1c ( $7.4 \pm 0.7$  to  $6.8 \pm 0.6$  and  $7.4 \pm 1.2$  to  $6.9 \pm 1.3$ , respectively) during six months. Only the low and middle groups showed a significant increment of active GLP-1, C-peptide level, a decreased log and proinsulin/insulin ratio after administration. In logistic analysis, the low or middle group is a significant

explanatory variable for an HbA1c decrease of  $\geq 0.5\%$ , and its odds ratio is 4.5 (1.40-17.6) ( $P = 0.01$ ) against the high active GLP-1 group. This remains independent when adjusted for HbA1c level before administration, patients' medical history, medications, insulin secretion and insulin resistance.

**CONCLUSION:** Plasma fasting active GLP-1 is an independent predictive marker for the efficacy of dipeptidyl peptidase 4 inhibitor sitagliptin.

**Key words:** Dipeptidyl peptidase-4 inhibitor; Active form glucagon-like peptide-1; Hemoglobin A1c; Regression analysis

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**Core tip:** This clinical trials study revealed novel non-responders for the sitagliptin treatment of patients with type 2 diabetes. The fasting active form of glucagon-like peptide-1 (GLP-1) is related to Hemoglobin A1c (HbA1c) lowering and is independent of the previously reported factors associated with non-responders, such as high body mass index or low baseline HbA1c. These non-responders did not show fasting active GLP-1 elevation after sitagliptin administration, nor following ameliorated beta cell function and insulin secretion. The mechanism of poor responsiveness is still not unveiled, however, measuring active GLP-1 might be a good marker for prognosis, and may help clarifying one aspect of response variation against sitagliptin.

Kushiyaama A, Kikuchi T, Tanaka K, Tahara T, Takao T, Onishi Y, Yoshida Y, Kawazu S, Iwamoto Y. Prediction of the effect on antihyperglycaemic action of sitagliptin by plasma active form glucagon-like peptide-1. *World J Diabetes* 2016; 7(11): 230-238 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v7/i11/230.htm> DOI: <http://dx.doi.org/10.4239/wjd.v7.i11.230>

## INTRODUCTION

Glucagon-like peptide-1 (GLP-1) is one of the major metabolic hormones<sup>[1]</sup>, so called incretins, that regulates glucose induced insulin secretion (GSIS)<sup>[2-4]</sup>. The active form of GLP-1 (active GLP-1) is secreted from intestinal L cells<sup>[5]</sup>, and dipeptidyl peptidase 4 (DPP-4) cuts N-terminal two amino acids of active GLP-1 into its inactive form rapidly in both type 2 diabetic patients and healthy subjects<sup>[6,7]</sup>. DPP-4 inhibitors retard GLP-1 degradation, raise plasma active GLP-1, and stimulate GSIS<sup>[8]</sup>. In patients with type 2 diabetes the effects of incretins are impaired, especially postprandially, when biologically intact active GLP-1 level is low<sup>[9]</sup>. DPP-4 inhibitors ameliorate active GLP-1 shortage, inhibit glucose spiking and help avoid hypoglycemia; therefore DPP-4 inhibitors are now widely used in the treatment of type 2 diabetes.

Sitagliptin<sup>[10]</sup> is one of the major selective DPP-4

inhibitors that improve glycemic control, both as a monotherapy and combined with other anti-hyperglycemic agents<sup>[11-15]</sup>. There have still been insufficient reports regarding predictors of the efficacy of DPP-4 inhibitor therapy. DPP-4 inhibitors appear to be more effective in patients with a high baseline HbA1c level<sup>[16-18]</sup>, low body mass index (BMI)<sup>[17,18]</sup>, low activity of plasma DPP-4<sup>[19]</sup>, in elderly patients and in patients displaying adequate compliance with diet/exercise therapy<sup>[20]</sup>. Therefore identifying the predictors of the therapeutic response to DPP-4 inhibitors would be valuable for its clinical use and help further speculation of the mechanism and pathophysiology of type 2 diabetes.

We hypothesized that the plasma level of active GLP-1 could be associated with the efficacy of DPP-4 inhibitors in patients with type 2 diabetes. Therefore we investigated the impact that baseline plasma active GLP-1 level had on HbA1c level after sitagliptin administration.

## MATERIALS AND METHODS

### Design and patients

This was an interventional single-arm study in patients with type 2 diabetes attending hospital at the Institute for Adult Diseases, Asahi Life Foundation, Tokyo, Japan. The protocol was approved by the Institutional Review Board (IRB) of the Institute for Adult Diseases, Asahi Life Foundation and was registered as clinical trial UMIN000010645. Patients with diabetes who attended the hospital's outpatient clinic were eligible to participate if they were  $\geq 20$  years old and had inadequate glycemic control [hemoglobin A1c (HbA1c)  $\geq 7.0\%$ ] despite dietary and exercise therapy and taking metformin and/or a sulfonylurea for at least three months.

Registration period: 24 mo from March 11, 2011. Follow-up period: 6 mo patient of final registration start treatment. The study period: The period plus the follow-up period to the registration period. All of the subjects gave written informed consent to be included in this study.

From these, adult patients (aged  $\geq 20$  years) with type 2 diabetes mellitus ( $n = 78$ ) were selected; and patients with type 1 diabetes, patients who took other DPP-4 inhibitors and/or a GLP-1 analog were excluded. Two patients were also excluded because their HbA1c level was below the lower limit of criteria at administration. Data collection was carried out as previously described<sup>[21]</sup>.

### Interventions

All 76 patients were given a 50 mg/d dose of sitagliptin, the standard dose for the treatment of type 2 diabetes in Japan, and were checked up with at monthly intervals for 6 mo, with at least two reviews in the first and third month. The doses of metformin and sulfonylurea were fixed throughout the 6-mo period, with a possible exception for the reduction of sulfonylurea when

avoiding anticipated hypoglycemia by doctors.

### Laboratory tests

Serum levels of active GLP-1 were measured with a commercially available enzyme-linked immunosorbent assay kit (#EGLP-36K, Merck Millipore, MA). Data collection of age, sex, disease duration, fasting blood glucose level, HbA1c level, BMI, medication/s taken, blood pressure, levels of biochemical indicators (liver function, renal function, uric acid, lipids) were carried out when starting sitagliptin administration. The estimated glomerular filtration rate (eGFR) was calculated using the estimation formula advocated by the Japanese Society of Nephrology:  $\text{eGFR (mL/min per } 1.73 \text{ m}^2) = 194 \times \text{Cr} - 1.094 \times \text{age} - 0.287 (\times 0.739 \text{ for women})^{[22]}$ .

The levels of plasma insulin (#SU06T, Fujirebio Inc., Tokyo, Japan), C peptide (#VU06T, Fujirebio Inc.), high sensitive C reactive protein (hsCRP) (#OQIY21, Siemens Healthcare, Erlangen, Germany), glucagon (#RB310, Euro-Diagnostica AB, Sweden) and proinsulin/immunoreactivity insulin ratio (PI/IRI) (#HPI-15K, Merck Millipore) were examined. HsCRP was evaluated as logarithmic. The measurement of HbA1c levels were carried out using HLC-723 GHb G8 analyzer (Tosoh Bioscience, Tokyo, Japan) as previously described<sup>[23]</sup>. During the third month of the administration period, levels of HbA1c, active GLP-1, insulin, C peptide, hsCRP, glucagon and the PI/IRI were again measured. As an index of efficacy, HbA1c decline (dA1c) was calculated during the 6-mo administration of sitagliptin, and a dA1c of  $\geq 0.5\%$  was considered effective.

### Statistical analysis

Subjects were divided into tertiles of high, medium and low active GLP-1 level prior to sitagliptin administration. To assess the statistical significance between groups, Tukey post hoc tests with ANOVA were performed, unless otherwise indicated.

Logistic analysis was used to examine whether active GLP-1, or any other factor, was the predictor of the efficacy of sitagliptin. In addition, using multivariate logistic analysis, we examined whether active GLP-1 is an independent predictor. The analysis was performed by Jmp 12.0.1 (SAS Institute, United States) and the data expressed in mean  $\pm$  SD. The data is illustrated in the table, boxplots and graphs; and  $P < 0.05$  is considered statistically significant.

## RESULTS

### Patients characteristics at baseline

In this study, 76 patients took 50 mg sitagliptin throughout the 6-mo period. No serious adverse effects, nor any adverse effects requiring a change or stop to medications were observed. Table 1 and Figure 1 indicate a patient profile of the subjects divided into three groups by their fasting active GLP-1 level. Measurements of active GLP-1

in the low active GLP-1 group were  $\leq 2$  pmol/L, less than assay sensitivity, and  $\geq 4$  pmol/L in the high group.

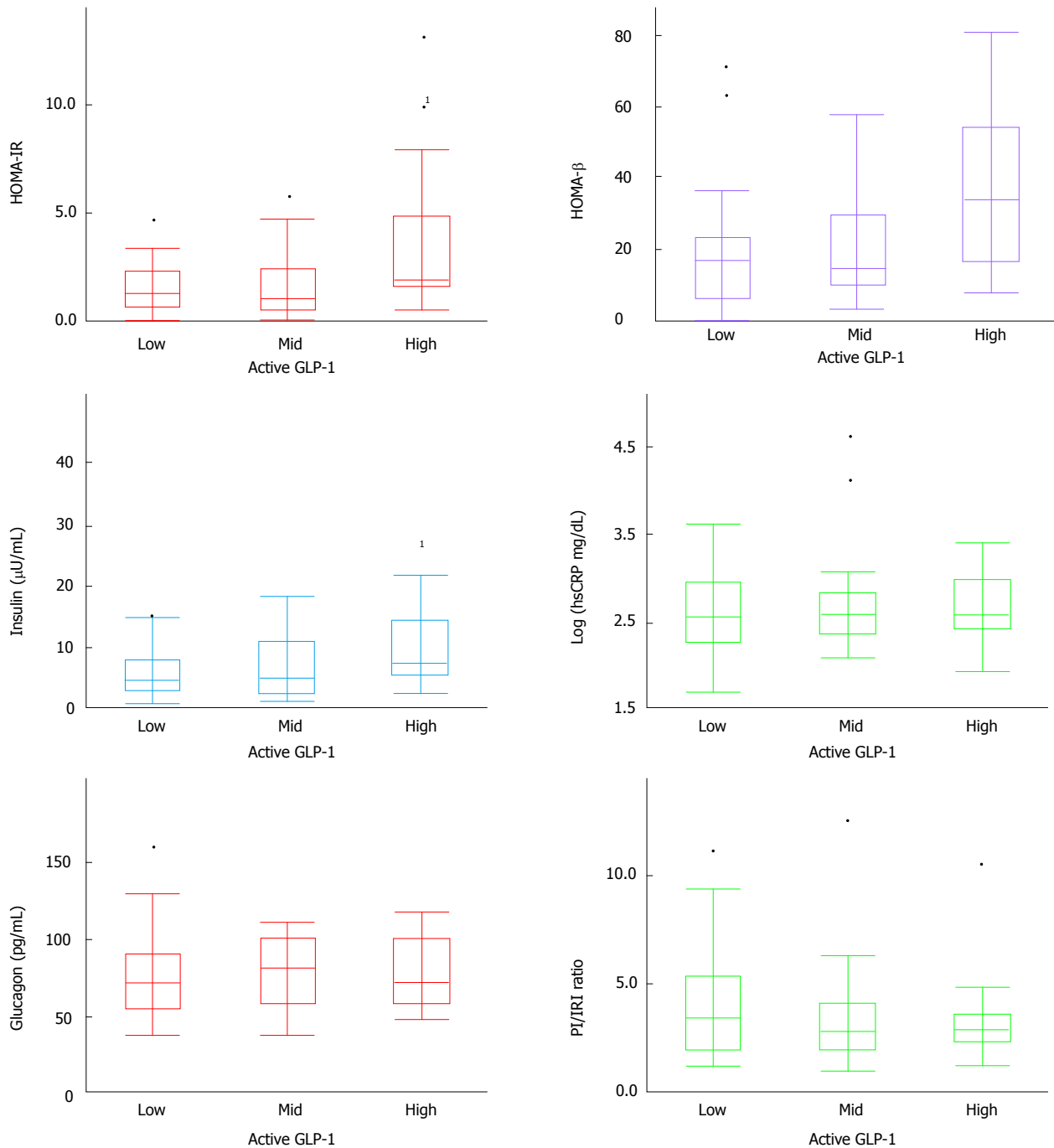
There was no significant difference when comparing sex, age, disease duration, glycemic control, and other parameters of serum profile between the high, middle and low active GLP-1 groups. However, high BMI and serum UA in the high active GLP-1 group was higher than other two groups. The frequency of Biguanide use rose with the increase of active GLP-1 level ( $P < 0.05$  Cochran-Armitage trend test). HOMA-IR and plasma insulin were significantly higher in the high active GLP-1 group compared with the other two groups (Figure 1). The high active GLP-1 group also tended to exhibit higher HOMA- $\beta$  and lower proinsulin/insulin ratio than two groups. There were no significant changes in hsCRP and plasma glucagon level between three groups.

As a result of sitagliptin administration, the high group did not show any decline of HbA1c ( $7.6\% \pm 1.4\%$  to  $7.5\% \pm 1.5\%$ ), whereas the middle and low indicated significant decline of HbA1c ( $7.4 \pm 0.7$  to  $6.8 \pm 0.6$  and  $7.4 \pm 1.2$  to  $6.9 \pm 1.3$ , respectively) during six months (Figure 2A). During the first three months of sitagliptin administration, the active GLP-1 level of the low group rose to detectable levels ( $\geq 2$  pmol/L). Likewise, the middle group showed a significant increment of active GLP-1, while the high group did not (Figure 2B).

Figure 3 indicates the changes of insulin, C-peptide, PI/insulin ratio, hsCRP, and glucagon for the low and middle groups against the high group over 3 mo, after the administration of sitagliptin. Insulin, C-peptide and PI/insulin levels in the low and middle groups were slightly increased, but tended to decrease in the high group. The changes of C-peptide and hsCRP during these three months were significant, yet fasting plasma glucagon level did not change between groups.

In logistic analysis, the low or middle active GLP-1 group is a significant explanatory variable for dA1c  $\geq 0.5\%$ , and its odds ratio (OR) is 4.5 (1.40-17.6) ( $P = 0.01$ ) when compared against the high active GLP-1 group (Table 2). High HbA1c, high fasting plasma glucose (FPG), high BMI, use of biguanide, high plasma insulin, high HOMA- $\beta$  and HOMA-IR at the beginning of administration of sitagliptin were also significant explanatory variables. Long disease duration is somewhat advantageous; while sex, age, use of sulfonylurea (SU), C-peptide level, PI/IRI, glucagon level, and hsCRP level at the beginning of administration were not significant.

To further investigate active GLP-1 level, independent, multivariate logistic analyses were performed (Table 2). Active GLP-1 level remained totally significant when adjusted with high HbA1c (model 1), and with HbA1c and background factors such as age, gender, disease duration and BMI (model 2). This was also observed with variables in model 2 and concomitant diabetes drugs (model 3), with model 2 variables and insulin and glucagon secretion, as well as inflammatory conditions (model 4), and also with variables in model 2 and HOMA-



**Figure 1** Baseline measurements of homeostasis model assessment of insulin resistance, HOMA-β, insulin, high sensitive C reactive protein, glucagon, and PI/insulin resistance index ratio. Patients' insulin resistance, insulin and glucagon secretion, and hsCRP at baseline is stratified against plasma active GLP-1 level. Data has been presented by boxplot. <sup>1</sup>Mean statistical significance  $P < 0.05$ . GLP-1: Glucagon-like peptide-1; hsCRP: High sensitive C reactive protein.

IR and HOMA-β (model 5).

## DISCUSSION

DPP-4 inhibitors show an indirect effect on glucose lowering through DPP-4 inhibition<sup>[24]</sup>, GLP-1 secretion, and subsequent GSIS and the inhibition of glucagon secretion. Therefore the effect of DPP-4 inhibitors is evaluated primarily by the inhibitory activity of DPP-4, and secondly by the postprandial increase of active GLP-1 concentrations; while significance of the basal

active GLP-1 value for the efficacy of DPP-4 inhibitor has been unknown.

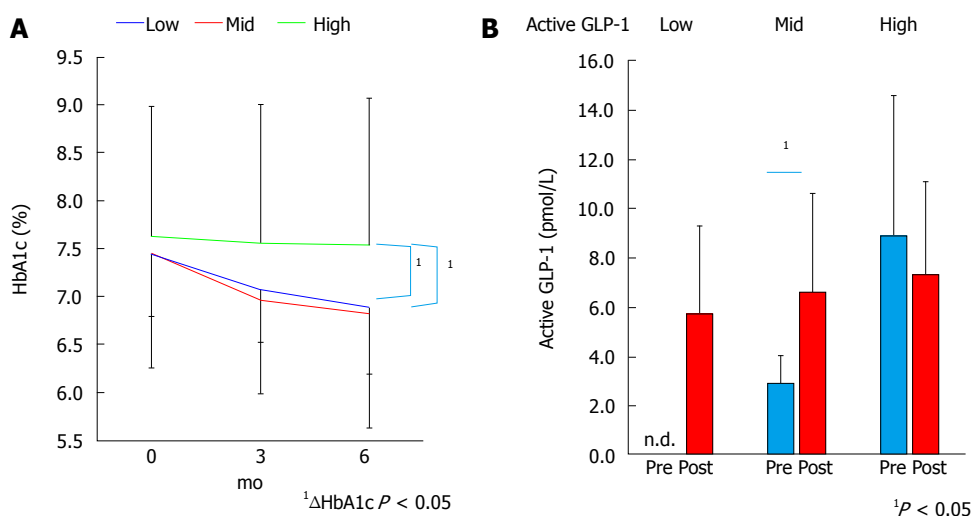
In this study, plasma active GLP-1 level of fasting is related to BMI, uric acid, the use of biguanide, HOMA-IR, HOMA-β, insulin level and PI/IRI ratio. The subjects in the high active GLP-1 group are characterized by insulin resistance, hyperinsulinemia and beta cell dysfunction. The high active GLP-1 group presented a decreased responsiveness in glucose lowering effect compared with the other two groups. Glucagon, commonly produced from preproglucagon<sup>[25]</sup>, is not related to active GLP-1



**Table 1 Patient characteristics (*n* = 76)**

	Active GLP-1 low ( <i>n</i> = 26)	Active GLP-1 mid ( <i>n</i> = 25)	Active GLP-1 high ( <i>n</i> = 25)	<i>P</i> value
Sex male/female (%)	82	88	88	
Age (yr)	60.5 ± 13.7	63.8 ± 10.8	58.5 ± 15.3	
Disease duration (yr)	16.2 ± 11.2	15.5 ± 10.8	12.7 ± 10.4	
FPG (mg/dL)	161.1 ± 35.4	160.1 ± 41.6	160.7 ± 45.3	
HbA1c (%)	7.43 ± 1.18	7.44 ± 0.66	7.61 ± 1.32	
BMI (kg/m <sup>2</sup> )	22.3 ± 5.8	24.1 ± 10.7	26.8 ± 5.6	<sup>1</sup>
Sulfonylurea (%)	54	52	57	
Biguanide (%)	45	52	76	
Systolic BP (mmHg)	126.8 ± 17.5	133.9 ± 15.1	129.2 ± 17.8	
Diastolic BP (mmHg)	74.5 ± 10.8	76.8 ± 13.6	78.0 ± 12.2	
Proteinuria	8	22	21	
γGTP (IU/L)	38.5 ± 34.3	45.9 ± 35.5	43.3 ± 27.5	
AST (IU/L)	28.3 ± 34.0	22.9 ± 8.2	25.0 ± 10.0	
ALT (IU/L)	36.0 ± 60.1	23.9 ± 15.8	32.2 ± 21.3	
TC (mg/dL)	193.3 ± 29.6	214.3 ± 32.0	189.1 ± 26.0	
HDLc (mg/dL)	57.0 ± 16.9	61.6 ± 16.0	51.9 ± 14.3	
TG (mg/dL)	127.1 ± 66.3	133.4 ± 132.0	135.1 ± 68.5	
LDLC (mg/dL)	109.8 ± 26.9	122.4 ± 36.5	110.0 ± 24.5	
UA (mg/dL)	4.87 ± 0.98	5.22 ± 1.09	5.65 ± 1.27	<sup>1</sup>
Cr (mg/dL)	0.83 ± 0.23	0.77 ± 0.15	0.75 ± 0.16	
eGFR (mL/min)	76.7 ± 21.3	78.8 ± 17.7	83.1 ± 19.3	

Data is presented as mean ± SD, or %. <sup>1</sup>Mean statistical significance *P* < 0.05. GLP-1: Glucagon-like peptide-1; HbA1c: Hemoglobin A1c; eGFR: Epidermal growth factor receptor; FPG: Fasting plasma glucose; BMI: Body mass index; GTP: Guanosine triphosphate; HDLC: High density lipoprotein cholesterol; LDLc: Low density lipoprotein cholesterol; AST: Glutamic-oxalacetic transaminase; ALT: Alanine aminotransferase; TG: Thyroglobulin; Cr: Chromium.



**Figure 2 Hemoglobin A1c and active glucagon-like peptide-1 change by sitagliptin administration.** A: Change of HbA1c by sitagliptin administration over six months, the data is presented in mean and S.D. <sup>1</sup>Statistical significance of change of HbA1c between the high active GLP-1 group and the low or middle groups; B: Active GLP-1 level before sitagliptin administration and after three months, stratified by plasma active GLP-1 level. Data is presented as the mean and S.D. <sup>1</sup>Mean statistical significance *P* < 0.05. GLP-1: Glucagon-like peptide-1; HbA1c: Hemoglobin A1c.

level. Insulin resistance is related to inflammation, and hsCRP reflects insulin resistance in some cases such as smokers or sufferers of polycystic ovarian syndrome<sup>[26,27]</sup>, however, hsCRP at baseline is not related to active GLP-1 level.

The factors defining plasma active GLP-1 level have not been reported, but are easily speculated as the balance between GLP-1 secretion and inactivation/degradation by DPP-4. If DPP-4 activity is low in insulin sensitive, non-obese subjects, low active GLP-1 level is probably derived from low GLP-1 secretion. In contrast,

insulin resistant patients indicated relatively high GLP-1 level in spite of presumably high DPP-4 activity<sup>[19]</sup>. Therefore, the reason the high baseline active GLP-1 group had the smallest response is probably due to the low contribution of the GLP-1 - DPP-4 system on their insufficient glycemic control or insulin action. The causes of this low contribution of GLP-1 - DPP-4 system should be focused on the fact that sitagliptin cannot raise GLP-1 level in the baseline high active GLP-1 group. One possible speculation is the insufficient inhibition of high DPP-4 activity by sitagliptin. In which

**Table 2** Logistic regression analysis to identify the factors associated with the efficacy of sitagliptin (dA1c  $\geq$  0.5%)

Variables	Univariate		Model 1		Model 2		Model 3		Model 4		Model 5	
	OR (95%CI)	P value	OR (95%CI)	P value	OR (95%CI)	P value	OR (95%CI)	P value	OR (95%CI)	P value	OR (95%CI)	P value
GLP-1 not high group	4.50 (1.40-17.6)	0.01	5.72 (1.64-25.99)	0.01	4.93 (1.07-32.96)	0.04	7.66 (1.48-57.48)	0.01	8.04 (1.30-75.83)	0.02	5.83 (1.12-45.6)	0.04
HbA1c (%)	1.81 (1.32-2.6)	0.001	1.69 (1.03-3.02)	0.04	2.30 (1.11-5.63)	0.02	2.23 (1.02-5.59)	0.05	2.63 (1.19-6.88)	0.02	2.51 (1.04-7.05)	0.04
FPG (mg/dL)	1.01 (1.00-1.02)	0.003										
Sex (male)	1.85 (0.73-4.65)	0.19			5.69 (0.73-57.54)	0.1	4.39 (0.51-47.1)	0.18	5.53 (0.46-92.72)	0.18	6.97 (0.78-90.5)	0.08
Age (yr)	1.00 (0.97-1.02)	0.77			0.98 (0.91-1.05)	0.53	0.96 (0.88-1.03)	0.26	0.99 (0.92-1.08)	0.89	0.97 (0.90-1.05)	0.48
Duration (yr)	1.03 (1.00-1.07)	0.09			1.01 (0.95-1.09)	0.73	1.01 (0.94-1.09)	0.77	1.03 (0.95-1.13)	0.44		0.67
BMI (kg/m <sup>2</sup> )	0.92 (0.83-0.99)	0.05			0.70 (0.50-0.91)	0.003	0.64 (0.43-0.86)	0.001	0.63 (0.39-0.90)	0.01	0.66 (0.43-0.92)	0.01
Sulfonylurea (+)	1.25 (0.63-2.46)	0.53					1.15 (0.22-6.13)	0.87				
Biguanide (+)	2.83 (1.42-5.76)	0.003					0.18 (0.02-1.01)	0.05				
Plasma insulin ( $\mu$ U/mL)	0.9 (0.82-0.98)	0.01							1.25 (0.94-1.69]	0.12		
C peptide (ng/mL)	0.8 (0.55-1.11)	0.19										
PI/IRI	1.33 (0.78-2.6)	0.29							4.94 (0.54-99.3)	0.17		
Glcagon (pg/mL)	0.99 (0.97-1.00)	0.11							0.97 (0.18-4.63)	0.97		
Log (hsCRP) [log (mg/mL)]	1.03 (0.53-1.98)	0.92							0.98 (0.94-1.01)	0.21		
HOMA- $\beta$	0.97 (0.95-0.99)	0.01									1.02 (0.94-1.09)	0.67
HOMA-IR	0.83 (0.67-0.99)	0.004									0.98 (0.45-2.15)	0.96

GLP-1: Glucagon-like peptide-1; HbA1c: Hemoglobin A1c; FPG: Fasting plasma glucose; BMI: Body mass index; hsCRP: High sensitive C reactive protein; PI/IRI: Proinsulin/immunoreactivity insulin ratio.

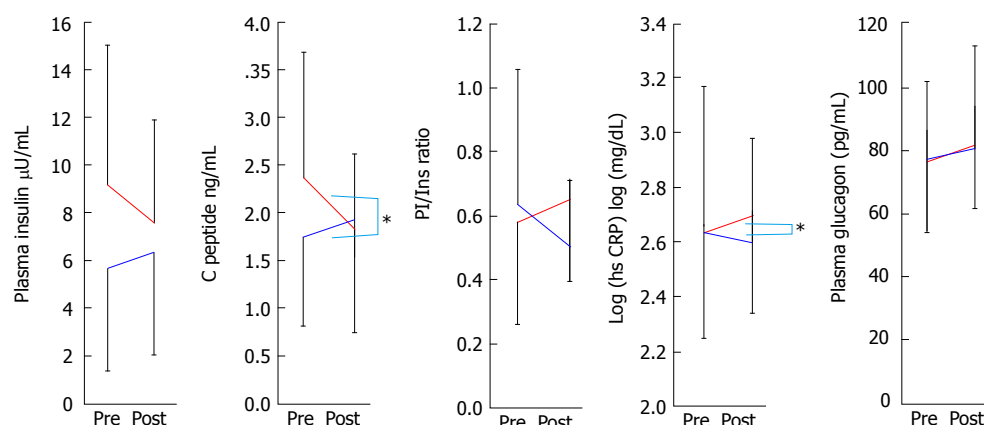
case, GLP-1 overcomes or evades high DPP-4 activity in insulin resistant subject. Activity of plasma DPP-4 correlates with insulin resistance and predicts sitagliptin efficacy<sup>[19]</sup>, however this was not measured. Another speculative cause is the unknown feedback regulation of active GLP-1 level other than DPP-4 activity, such as incretion from L cells. Injection of excessive GLP-1 can cause nausea or vomiting more frequently than administration of DPP-4 inhibitors<sup>[28]</sup>. Therefore there might be a physiological cap of GLP-1 level caused by unknown factors other than DPP-4, thus avoiding the imbalance of gastrointestinal homeostasis or other catastrophe.

Aside from the result of examination of multiple regression, it is clearly demonstrated that active GLP-1 is statistically independent of other factors, such as HbA1c, disease history, use of medications, the specific hormonal parameters for insulin, glucagon, low-grade inflammation, and HOMA indicators. Active GLP-1 level correlated with insulin resistance but predicts HbA1c improvement independently to insulin resistance. The high active GLP-1 and high DPP-4 activity from insulin

resistance might have an additive effect on resistance to sitagliptin treatment.

In accordance with previous reports, our results show the significant predictive capabilities of HbA1c improvement due to sitagliptin treatment, such as high baseline HbA1c, and low BMI<sup>[17,18,29]</sup>. The positive relationship between baseline HbA1c and the magnitude of HbA1c change by glucose-lowering therapies was irrespective of class or mode of action of therapy category<sup>[30]</sup>. In addition to BMI, several negative predictive variables are shown; uric acid and high HOMA-IR are pathophysiologically derived from insulin resistance. Hyperinsulinemia and high HOMA- $\beta$  are also speculated to be subsequent or a compensatory result of insulin resistance, and the use of biguanide is an arbitrary selection of medication for insulin resistant patients. Biguanide itself is reported to increase active GLP-1<sup>[31]</sup> and is effective<sup>[32]</sup> in combination with sitagliptin.

Other estimations for long term glycemic management were previously stated, by means of short-term response of change of C-peptide immunoreactivity index<sup>[33]</sup> or glycated HbA1c<sup>[34]</sup>. Our data indicated similar findings for insulin secretion and HbA1c change over



**Figure 3** Change in insulin and glucagon secretion, high sensitive C reactive protein during three months of sitagliptin administration stratified against plasma active glucagon-like peptide-1 level at baseline. The data is presented as mean and S.D. \*statistical significance of change of HbA1c between the high active GLP-1 group and the low or middle groups. GLP-1: Glucagon-like peptide-1; HbA1c: Hemoglobin A1c.

three months, and furthermore, the change in hsCRP is associated with baseline active GLP-level. It was already documented that a significant inverse correlation was found between changes in GLP-1 and changes in CRP levels<sup>[35]</sup>. Those predictors seem useful also when anticipating long term effects over a short period, although these changes cannot be predicted before administration. It was also demonstrated that compliance with diet/exercise therapy, weight gain<sup>[20]</sup>, and increased polyunsaturated free fatty acid (eicosapentanoic acid and docosahexanoic acid) level from fish intake<sup>[36]</sup> predicted the efficacy of sitagliptin. The relevance of the predictors, such as compliance with diet/exercise therapy, weight gain and polyunsaturated fatty acid consumption, in relation to fasting active GLP-1 levels is not clear.

It was reported that GLP-1 levels decreased in Caucasian diabetes patients when compared to non-diabetic subjects<sup>[37]</sup>, GLP-1 levels were much lower in Japanese patients who tend to have lower insulinogenic capabilities compared to Caucasians<sup>[38]</sup>. This low level of GLP-1 is a risk factor of diabetes onset<sup>[39]</sup>. Therefore, sitagliptin is probably adequate or effective for low GLP-1 patients and for lower insulinogenic ethnicities such as Japanese. However, our recent report shows young Japanese diabetics tend to be obese and might have higher insulin resistance than previously considered<sup>[23]</sup>, these pathophysiological changes in Japanese patients might decrease the effectiveness of sitagliptin.

This study has several limitations. Firstly, one third of subjects exhibited levels below sensitivity parameters. When assay sensitivity has been improved, they might be further classified. Other limitations are the design, the study of an open-label, single arm trial and the somewhat small spectrum of subjects, and it being a single-ethnicity study, performed in a single health center. In addition, inactive GLP-1, postprandial GLP-1 and DPP-4 activity were not measured, which may have been helpful to resolve the remaining questions from the study.

As announced in TECOS trial<sup>[40,41]</sup> and in another

cohort study<sup>[42]</sup>, sitagliptin is safe in regards to the development of cardiovascular events, and is a useful agent that can significantly reduce HbA1c. However, sitagliptin does not greatly exceed traditional treatments with respect to this HbA1c lowering effect<sup>[40]</sup>. Thus it is important to avoid applying this treatment to subjects supposed to be non-responders. In spite of the limitations above, this examination was successful in determining whether a patient is to be given sitagliptin or not, using only a single collection of blood. Measuring active GLP-1 in fasting plasma can give another evaluation of the characteristics of patients with type 2 diabetes, independent of insulin secretion and insulin resistance. For daily practical use, the examination costs were rather expensive and health insurance does not apply to this in Japan, and a standard test should be confirmed as a worldwide standard.

In conclusion, we discovered a new factor that predicts the efficiency of sitagliptin, fasting active GLP-1.

## COMMENTS

### Background

Glucagon-like peptide-1 (GLP-1) regulates glucose induced insulin secretion. Dipeptidyl peptidase-4 (DPP-4) inactivates the active form of GLP-1. Therefore DPP-4 inhibitors retard GLP-1 degradation, raise plasma active GLP-1, and stimulate glucose induced insulin secretion (GSIS). DPP-4 inhibitors are now widely used in the treatment of type 2 diabetes. Sitagliptin is one of the major selective DPP-4 inhibitors.

### Research frontiers

Sitagliptin is the most frequently used DPP-4 inhibitor, however not enough is known about the predictors of this therapeutic response. Identifying the predictors would be valuable for its clinical use and help further speculation of the mechanism and pathophysiology of type 2 diabetes. The authors hypothesized that the plasma level of active GLP-1 could be associated with the efficacy of DPP-4 inhibitors in patients with type 2 diabetes.

### Innovations and breakthroughs

The subjects in the high active GLP-1 group are characterized by insulin resistance. Those subjects are newly founded non-responders for sitagliptin treatment. The active GLP-1 level and insulin secretion of the subjects rose

only in low and middle active GLP-1 groups, while those in high group did not.

### Applications

Sitagliptin is probably adequate or effective for low GLP-1 patients and for lower insulinogenic ethnicities such as Japanese. However, the recent report shows young Japanese diabetics tend to be obese and might have higher insulin resistance than previously considered, these pathophysiological changes in Japanese patients might decrease the effectiveness of sitagliptin.

### Terminology

GLP-1, glucagon like peptide-1, is one of the major metabolic hormones, so called incretins. GLP-1 regulates glucose induced insulin secretion. The active form of GLP-1 (active GLP-1) is secreted from intestinal L cells, and DPP-4 cuts N-terminal two amino acids of active GLP-1 into its inactive form rapidly. DPP-4 inhibitors retard GLP-1 degradation, raise plasma active GLP-1, and stimulate GSIS. In patients with type 2 diabetes, the effects of incretins are impaired, especially postprandially, when biologically intact active GLP-1 level is low. DPP-4 inhibitors ameliorate active GLP-1 shortages, inhibit glucose spiking and help avoid hypoglycemia.

### Peer-review

This was a well conducted study that has clinical implications. Overall, this study makes an important observation regarding the prediction of the efficacy of DPP-4 inhibitor-therapy based on a baseline clinical parameter.

## REFERENCES

- Bell GI, Santerre RF, Mullenbach GT. Hamster preproglucagon contains the sequence of glucagon and two related peptides. *Nature* 1983; **302**: 716-718 [PMID: 6835407 DOI: 10.1038/302716a0]
- Brandt A, Katschinski M, Arnold R, Polonsky KS, Göke B, Byrne MM. GLP-1-induced alterations in the glucose-stimulated insulin secretory dose-response curve. *Am J Physiol Endocrinol Metab* 2001; **281**: E242-E247 [PMID: 11440899]
- Holst JJ, Orskov C, Nielsen OV, Schwartz TW. Truncated glucagon-like peptide I, an insulin-releasing hormone from the distal gut. *FEBS Lett* 1987; **211**: 169-174 [PMID: 3542566 DOI: 10.1016/0014-5793(87)81430-8]
- Zander M, Madsbad S, Madsen JL, Holst JJ. Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study. *Lancet* 2002; **359**: 824-830 [PMID: 11897280 DOI: 10.1016/S0140-6736(02)07952-7]
- Mojsov S, Heinrich G, Wilson IB, Ravazzola M, Orci L, Habener JF. Preproglucagon gene expression in pancreas and intestine diversifies at the level of post-translational processing. *J Biol Chem* 1986; **261**: 11880-11889 [PMID: 3528148]
- Mentlein R, Gallwitz B, Schmidt WE. Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7-36)amide, peptide histidine methionine and is responsible for their degradation in human serum. *Eur J Biochem* 1993; **214**: 829-835 [PMID: 8100523 DOI: 10.1111/j.1432-1033.1993.tb17986.x]
- Deacon CF, Nauck MA, Toft-Nielsen M, Pridal L, Willms B, Holst JJ. Both subcutaneously and intravenously administered glucagon-like peptide I are rapidly degraded from the NH2-terminus in type II diabetic patients and in healthy subjects. *Diabetes* 1995; **44**: 1126-1131 [PMID: 7657039 DOI: 10.2337/diabetes.50.7.1588]
- Deacon CF, Danielsen P, Klarskov L, Olesen M, Holst JJ. Dipeptidyl peptidase IV inhibition reduces the degradation and clearance of GIP and potentiates its insulinotropic and antihyperglycemic effects in anesthetized pigs. *Diabetes* 2001; **50**: 1588-1597 [PMID: 11423480]
- Vilsbøll T, Krarup T, Deacon CF, Madsbad S, Holst JJ. Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in type 2 diabetic patients. *Diabetes* 2001; **50**: 609-613 [PMID: 11246881 DOI: 10.2337/diabetes.50.3.609]
- Kim D, Wang L, Beconi M, Eiermann GJ, Fisher MH, He H, Hickey GJ, Kowalchick JE, Leitig B, Lyons K, Marsilio F, McCann ME, Patel RA, Petrov A, Scapin G, Patel SB, Roy RS, Wu JK, Wyvratt MJ, Zhang BB, Zhu L, Thornberry NA, Weber AE. (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine: a potent, orally active dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. *J Med Chem* 2005; **48**: 141-151 [PMID: 15634008 DOI: 10.1021/jm0493156]
- Ohmura H, Mita T, Taneda Y, Sugawara M, Funayama H, Matsuoka J, Watada H, Daida H. Efficacy and safety of sitagliptin in Japanese patients with type 2 diabetes. *J Clin Med Res* 2015; **7**: 211-219 [PMID: 25699116 DOI: 10.14740/jocmr1983w]
- Hsieh CJ, Shen FC. The durability of sitagliptin in elderly patients with type 2 diabetes. *Clin Interv Aging* 2014; **9**: 1905-1911 [PMID: 25422588 DOI: 10.2147/CIA.S72396]
- Henry RR, Staels B, Fonseca VA, Chou MZ, Teng R, Golm GT, Langdon RB, Kaufman KD, Steinberg H, Goldstein BJ. Efficacy and safety of initial combination treatment with sitagliptin and pioglitazone--a factorial study. *Diabetes Obes Metab* 2014; **16**: 223-230 [PMID: 23909985 DOI: 10.1111/dom.12194]
- Iwamoto Y, Tajima N, Kadowaki T, Nonaka K, Taniguchi T, Nishii M, Arjona Ferreira JC, Amatruda JM. Efficacy and safety of sitagliptin monotherapy compared with voglibose in Japanese patients with type 2 diabetes: a randomized, double-blind trial. *Diabetes Obes Metab* 2010; **12**: 613-622 [PMID: 20590736 DOI: 10.1111/j.1463-1326.2010.01197.x]
- Williams-Herman D, Johnson J, Teng R, Golm G, Kaufman KD, Goldstein BJ, Amatruda JM. Efficacy and safety of sitagliptin and metformin as initial combination therapy and as monotherapy over 2 years in patients with type 2 diabetes. *Diabetes Obes Metab* 2010; **12**: 442-451 [PMID: 20415693 DOI: 10.1111/j.1463-1326.2010.01204.x]
- Barzilai N, Guo H, Mahoney EM, Caporossi S, Golm GT, Langdon RB, Williams-Herman D, Kaufman KD, Amatruda JM, Goldstein BJ, Steinberg H. Efficacy and tolerability of sitagliptin monotherapy in elderly patients with type 2 diabetes: a randomized, double-blind, placebo-controlled trial. *Curr Med Res Opin* 2011; **27**: 1049-1058 [PMID: 21428727 DOI: 10.1185/03007995.2011.568059]
- Chung HS, Suh S, Kim MY, Kim SK, Kim HK, Lee JI, Hur KY, Kim JH, Min YK, Lee MS, Kim KW, Kim SW, Chung JH, Lee MK. Predictive factors of durability to sitagliptin: Slower reduction of glycated hemoglobin, older age and higher baseline glycated hemoglobin. *J Diabetes Investig* 2014; **5**: 51-59 [PMID: 24843737 DOI: 10.1111/jdi.12127]
- Ishikawa M, Takai M, Maeda H, Kanamori A, Kubota A, Amemiya H, Iizuka T, Iemitsu K, Iwasaki T, Uehara G, Umezawa S, Obana M, Kaneshige H, Kaneshiro M, Kawata T, Sasai N, Saito T, Takuma T, Takeda H, Tanaka K, Tsurui N, Nakajima S, Hoshino K, Honda S, Machimura H, Matoba K, Minagawa F, Minami N, Miyairi Y, Mokubo A, Motomiya T, Waseda M, Miyakawa M, Naka Y, Terauchi Y, Tanaka Y, Matsuba I. Factors Predicting Therapeutic Efficacy of Combination Treatment With Sitagliptin and Insulin in Type 2 Diabetic Patients: The ASSIST-K Study. *J Clin Med Res* 2015; **7**: 607-612 [PMID: 26124906 DOI: 10.14740/jocmr1249w]
- Aso Y, Ozeki N, Terasawa T, Naruse R, Hara K, Suetsugu M, Takebayashi K, Shibazaki M, Haruki K, Morita K, Inukai T. Serum level of soluble CD26/dipeptidyl peptidase-4 (DPP-4) predicts the response to sitagliptin, a DPP-4 inhibitor, in patients with type 2 diabetes controlled inadequately by metformin and/or sulfonylurea. *Transl Res* 2012; **159**: 25-31 [PMID: 22153807 DOI: 10.1016/j.trsl.2011.09.005]
- Kanamori A, Matsuba I. Factors associated with reduced efficacy of sitagliptin therapy: analysis of 93 patients with type 2 diabetes treated for 1.5 years or longer. *J Clin Med Res* 2013; **5**: 217-221 [PMID: 23671547 DOI: 10.4021/jocmr1256w]
- Tanaka K, Hara S, Hattori M, Sakai K, Onishi Y, Yoshida Y, Kawazu S, Kushiyaama A. Role of elevated serum uric acid levels at the onset of overt nephropathy in the risk for renal function decline in patients with type 2 diabetes. *J Diabetes Investig* 2015; **6**: 98-104 [PMID: 25621139 DOI: 10.1111/jdi.12243]



- 22 **Matsuo S**, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, Yamagata K, Tomino Y, Yokoyama H, Hishida A. Revised equations for estimated GFR from serum creatinine in Japan. *Am J Kidney Dis* 2009; **53**: 982-992 [PMID: 19339088 DOI: 10.1053/j.ajkd.2008.12.034]
- 23 **Kushiyama A**, Yoshida Y, Kikuchi T, Suzawa N, Yamamoto M, Tanaka K, Okayasu M, Tahara T, Takao T, Onishi Y, Kawazu S. Twenty-year trend of increasing obesity in young patients with poorly controlled type 2 diabetes at first diagnosis in urban Japan. *J Diabetes Investig* 2013; **4**: 540-545 [PMID: 24843707 DOI: 10.1111/jdi.12090]
- 24 **Gibbs JP**, Fredrickson J, Barbee T, Correa I, Smith B, Lin SL, Gibbs MA. Quantitative model of the relationship between dipeptidyl peptidase-4 (DPP-4) inhibition and response: meta-analysis of alogliptin, saxagliptin, sitagliptin, and vildagliptin efficacy results. *J Clin Pharmacol* 2012; **52**: 1494-1505 [PMID: 22162539 DOI: 10.1177/0091270011420153]
- 25 **Bell GI**, Sanchez-Pescador R, Laybourn PJ, Najarian RC. Exon duplication and divergence in the human preproglucagon gene. *Nature* 1983; **304**: 368-371 [PMID: 6877358 DOI: 10.1038/304368a0]
- 26 **Hanyu O**, Yoshida J, Abe E, Hirayama S, Miyake K, Aizawa Y, Miida T. High-sensitivity CRP reflects insulin resistance in smokers. *J Atheroscler Thromb* 2009; **16**: 560-567 [PMID: 19749491 DOI: 10.5551/jat.869]
- 27 **Bahceci M**, Tuzcu A, Canoruc N, Tuzun Y, Kidir V, Aslan C. Serum C-reactive protein (CRP) levels and insulin resistance in non-obese women with polycystic ovarian syndrome, and effect of bicalutamide on hirsutism, CRP levels and insulin resistance. *Horm Res* 2004; **62**: 283-287 [PMID: 15542929 DOI: 10.1159/000081973]
- 28 **Drucker DJ**, Nauck MA. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet* 2006; **368**: 1696-1705 [PMID: 17098089 DOI: 10.1016/S0140-6736(06)69705-5]
- 29 **Kim SA**, Shim WH, Lee EH, Lee YM, Beom SH, Kim ES, Yoo JS, Nam JS, Cho MH, Park JS, Ahn CW, Kim KR. Response: predictive clinical parameters for the therapeutic efficacy of sitagliptin in Korean type 2 diabetes mellitus (diabetes metab j 2011; 35: 159-65). *Diabetes Metab J* 2011; **35**: 300-301 [PMID: 21785752 DOI: 10.4093/dmj.2011.35.3.300]
- 30 **DeFronzo RA**, Stonehouse AH, Han J, Wintle ME. Relationship of baseline HbA1c and efficacy of current glucose-lowering therapies: a meta-analysis of randomized clinical trials. *Diabet Med* 2010; **27**: 309-317 [PMID: 20536494 DOI: 10.1111/j.1464-5491.2010.02941.x]
- 31 **Wu T**, Thazhath SS, Bound MJ, Jones KL, Horowitz M, Rayner CK. Mechanism of increase in plasma intact GLP-1 by metformin in type 2 diabetes: stimulation of GLP-1 secretion or reduction in plasma DPP-4 activity? *Diabetes Res Clin Pract* 2014; **106**: e3-e6 [PMID: 25172519 DOI: 10.1016/j.diabres.2014.08.004]
- 32 **Yokoh H**, Kobayashi K, Sato Y, Takemoto M, Uchida D, Kanatsuka A, Kuribayashi N, Terano T, Hashimoto N, Sakurai K, Hanaoka H, Ishikawa K, Onishi S, Yokote K. Efficacy and safety of the dipeptidyl peptidase-4 inhibitor sitagliptin compared with alpha-glucosidase inhibitor in Japanese patients with type 2 diabetes inadequately controlled on metformin or pioglitazone alone (Study for an Ultimate Combination Therapy to Control Diabetes with Sitagliptin-1): A multicenter, randomized, open-label, non-inferiority trial. *J Diabetes Investig* 2015; **6**: 182-191 [PMID: 25802726 DOI: 10.1111/jdi.12282]
- 33 **Nishimura T**, Meguro S, Sekioka R, Tanaka K, Saisho Y, Irie J, Tanaka M, Kawai T, Itoh H. C-peptide immunoreactivity index is associated with improvement of HbA1c: 2-Year follow-up of sitagliptin use in patients with type 2 diabetes. *Diabetes Res Clin Pract* 2015; **108**: 441-447 [PMID: 25795273 DOI: 10.1016/j.diabres.2015.02.031]
- 34 **Hamaguchi T**, Koga M, Murai J, Saito H, Tamada D, Kurebayashi S, Katsuno T, Miyagawa J, Namba M. Estimation of HbA1c response to sitagliptin by change in glycated albumin level for 2 weeks. *J Diabetes Investig* 2012; **3**: 175-178 [PMID: 24843562 DOI: 10.1111/j.2040-1124.2011.00167.x]
- 35 **Tremblay AJ**, Lamarche B, Deacon CF, Weisnagel SJ, Couture P. Effects of sitagliptin therapy on markers of low-grade inflammation and cell adhesion molecules in patients with type 2 diabetes. *Metabolism* 2014; **63**: 1141-1148 [PMID: 25034387 DOI: 10.1016/j.metabol.2014.06.004]
- 36 **Iwasaki M**, Hoshian F, Tsuji T, Hirose N, Matsumoto T, Kitatani N, Sugawara K, Usui R, Kuwata H, Sugizaki K, Kitamoto Y, Fujiwara S, Watanabe K, Hyo T, Kurose T, Seino Y, Yabe D. Predicting efficacy of dipeptidyl peptidase-4 inhibitors in patients with type 2 diabetes: Association of glycated hemoglobin reduction with serum eicosapentaenoic acid and docosahexaenoic acid levels. *J Diabetes Investig* 2012; **3**: 464-467 [PMID: 24843607 DOI: 10.1111/j.2040-1124.2012.00214.x]
- 37 **Vilsbøll T**, Agersø H, Krarup T, Holst JJ. Similar elimination rates of glucagon-like peptide-1 in obese type 2 diabetic patients and healthy subjects. *J Clin Endocrinol Metab* 2003; **88**: 220-224 [PMID: 12519856 DOI: 10.1210/jc.2002-021053]
- 38 **Seino Y**, Fukushima M, Yabe D. GIP and GLP-1, the two incretin hormones: Similarities and differences. *J Diabetes Investig* 2010; **1**: 8-23 [PMID: 24843404 DOI: 10.1111/j.2040-1124.2010.00022.x]
- 39 **Lastya A**, Saraswati MR, Suastika K. The low level of glucagon-like peptide-1 (glp-1) is a risk factor of type 2 diabetes mellitus. *BMC Res Notes* 2014; **7**: 849 [PMID: 25427660 DOI: 10.1186/1756-0500-7-849]
- 40 **Green JB**, Bethel MA, Armstrong PW, Buse JB, Engel SS, Garg J, Josse R, Kaufman KD, Koglin J, Korn S, Lachin JM, McGuire DK, Pencina MJ, Standl E, Stein PP, Suryawanshi S, Van de Werf F, Peterson ED, Holman RR. Effect of Sitagliptin on Cardiovascular Outcomes in Type 2 Diabetes. *N Engl J Med* 2015; **373**: 232-242 [PMID: 26052984 DOI: 10.1056/NEJMoa1501352]
- 41 **Green JB**, Bethel MA, Paul SK, Ring A, Kaufman KD, Shapiro DR, Califf RM, Holman RR. Rationale, design, and organization of a randomized, controlled Trial Evaluating Cardiovascular Outcomes with Sitagliptin (TECOS) in patients with type 2 diabetes and established cardiovascular disease. *Am Heart J* 2013; **166**: 983-989. e7 [PMID: 24268212 DOI: 10.1016/j.ahj.2013.09.003]
- 42 **Wang SH**, Chen DY, Lin YS, Mao CT, Tsai ML, Hsieh MJ, Chou CC, Wen MS, Wang CC, Hsieh IC, Hung KC, Chen TH. Cardiovascular Outcomes of Sitagliptin in Type 2 Diabetic Patients with Acute Myocardial Infarction, a Population-Based Cohort Study in Taiwan. *PLoS One* 2015; **10**: e0131122 [PMID: 26115092 DOI: 10.1371/journal.pone.0131122]

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## Blunting post-meal glucose surges in people with diabetes

Elsamma Chacko

Elsamma Chacko, Connecticut Valley Hospital, Middletown, CT 06457, United States

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**Correspondence to:** Elsamma Chacko, Principal Physician, Connecticut Valley Hospital, 1000 Silver Street, Middletown, CT 06457, United States. [elsammac@msn.com](mailto:elsammac@msn.com)  
 Telephone: +1-860-2625000  
 Fax: +1-860-2625307

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### Abstract

Worldwide, the morbidity and mortality associated with non-communicable diseases have been climbing steadily - with costs aggressively keeping pace. This letter highlights a decidedly low-cost way to address the challenges posed by diabetes. High levels of postprandial blood glucose are disproportionately linked to much of the microvascular damage which, in the end, leads to macrovascular complications and organ failures. Systematically controlling post-meal glucose surges is a critical element of overall glycemic management in

diabetes. Diet, exercise and medications form a triad of variables that individuals engaged in diabetes self-management may manipulate to achieve their targeted glucose levels. As a rule, diabetes patients in developing countries as well as those living in the pockets of poverty in the western world cannot afford special diets, medications, glucometers and supplies, lab tests and office visits. Exercise is the one option that is readily accessible to all. Decades of research in laboratory settings, viewed holistically, have established that light to moderate aerobic exercise for up to 60 min starting 30 min after the first bite into a meal can blunt the ensuing glucose surge effectively. Moderate resistance exercise, moderate endurance exercise or a combination of the two, practiced post-meal has also been found to improve many cardio-metabolic markers: Glucose, high density lipoprotein, triglycerides, and markers of oxidative stress. On the other hand, pre-breakfast exercise and high-intensity exercise in general have been decidedly counterproductive.

**Key words:** Pre-meal exercise; Post-meal exercise; Exercise timing; Exercise intensity; Glucose surge; Insulin resistance

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**Core tip:** A critical part of diabetes self-management is the systematic blunting of the post-meal glucose surge. The reason for this is that the glucose surge is closely linked to the vascular complications of diabetes and eventual organ damage. Decades of studies have shown that a moderate intensity exercise - aerobic, resistance or combined - starting 30-40 min after the start of the meal can efficiently blunt the glucose peak. Post-meal studies starting at other times have also shown improvements in other metabolic markers including high density lipoprotein, triglycerides and markers of oxidative stress. Promoting post-meal exercise can make a big difference in the daily lives of diabetes patients worldwide.

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## TO THE EDITOR

Non-communicable chronic diseases are costly<sup>[1]</sup>. The public at large foots a sizable chunk of the economic cost while patients are faced with the double whammy of compromised general health and sizable monitory costs. This note highlights a surprisingly low-cost way to address this problem worldwide.

Moderating post-meal glucose surges is a critical part of diabetes management because these surges are directly linked to the microvascular complications that in turn lead to organ damage and increased morbidity and mortality<sup>[2]</sup>. There are three well recognized approaches to managing post-meal glucose peaks: Diet, exercise and medications. Poverty, however, stands in the way of using food and medications properly to fight post-meal glucose surges. Physical activity is free for all.

People with insulin resistance have difficulty processing carbohydrates. The general recommendation for getting around this problem has been to rely on "appropriate" carbohydrate intake. American Diabetes Association (ADA) recommends individualized meal plans with the right carb count that would offer a postprandial glucose (ppg) value under 180 mg/dL (9.99 mmol/L)<sup>[3]</sup> with an expected HbA1c of 7.0%. American Association of Clinical Endocrinologists (AACE) is even more cautious: AACE recommends a ppg under 140 mg/dL (7.77 mmol/L) and an expected HbA1c of 6.5%<sup>[4]</sup>. Since glucose levels peak around 1 h after the start of meals<sup>[5]</sup> patients who have glucometers should be able to adjust the carb content of meals with the help of the 1-h glucose value following the major meal of the day. Balancing meals with protein, vegetables, fiber and healthy fat decreases the glycemic load of the meal and offers lower glucose peaks<sup>[6,7]</sup>.

Studies featuring moderate exercise after meals consistently show glucose levels going down. It is possible to blunt the post-meal glucose surge substantially by starting the physical activity about 30 min post-meal and continuing it for up to 60 min<sup>[8-11]</sup>. This enables the body to use up the incoming glucose molecules to do the work involved in the activity - before they get to build up into a big peak. Insulin levels go up following meals, hepatic glucose production is suppressed and the meal-derived glucose gets used up preferentially as fuel<sup>[12]</sup>. Hypoglycemia is not of concern during this period<sup>[8]</sup>. Symptomatic exercise-induced hypoglycemia occurs rarely when the activity is performed during the late postprandial period, two hours or more after the meal<sup>[13]</sup>. Pre-meal exercise, on the other hand, increases postprandial glucose surges<sup>[9,14-17]</sup> although glucose is fairly steady for the duration of the pre-meal exercise itself<sup>[18-20]</sup>. The post-

exercise glucose elevation is even more pronounced in the case of high-intensity exercise<sup>[21-24]</sup>. Pre-breakfast exercise is fueled mainly by hepatic glucose and at the end of the exercise bout the excess glucose arriving from the liver accumulates in the blood, resulting in a post-exercise glucose elevation<sup>[21-24]</sup>. If lowering the post-meal glucose peak is the goal, pre-meal exercise is the wrong thing to do. A brisk walk for 30 min after the start of every major meal is one option. If walking three times a day is too much, one may opt for one major meal, preferably breakfast, along with smaller meals the rest of the day.

Moderate resistance exercise for 45 min at 45 min post-meal also lowered glucose levels, partially blunting the glucose peak<sup>[25]</sup>. When post-meal resistance exercise was combined with endurance exercise, hyperglycemia was reduced for the subsequent 24 h by 39%<sup>[26]</sup>. Moderate post-meal exercise, resistance or aerobic or a combination, improved other metabolic markers also: lipids and markers of oxidative stress<sup>[25,27-30]</sup>. It looks like moderate resistance and aerobic activities decrease glucose levels directly during the exercise by increasing glucose transport out of the bloodstream. Moderate resistance exercise also improves insulin sensitivity for 24 h or more after the exercise bout.

Taken together, the available data point to 30 min post-meal as the optimal point to start the exercise activity. The mode of exercise can be resistance, aerobic or a combination at moderate intensity. The guidelines recommend resistance exercise 3 times a week<sup>[3,4]</sup>. There are also other health benefits -mainly physical fitness and body composition - to be had by doing resistance before endurance to minimize the interference effect<sup>[31]</sup>. The aerobic activity can include a brisk walk, treadmill, elliptical, rowing, stationary bike, dancing or swimming. Resistance exercise can be a 10 min workout using free weights involving major muscle groups. It is important to keep the intensity below 80%  $\text{VO}_{2\text{max}}$ . People under free living conditions have at least three ways to keep the right intensity. They may exercise at a pace that causes a slight shortness of breath. They may also keep  $\text{HR}_{\text{max}}$  at 60%-70%. The maximum heart rate,  $\text{HR}_{\text{max}}$ , is calculated as  $220 - \text{age}$  (For example, the  $\text{HR}_{\text{max}}$  for a 40-year-old is 180 beats/min; the corresponding pulse rate during the physical activity should be 104-126 beats/min). Those who have glucometers may also check glucose at the end of the workout and adjust the intensity accordingly for the next session.

When it comes to medications, various classes of drugs are available today specifically to manage post-meal glucose surges. These include glinides, short-acting insulins, gliptins, DPP-4 inhibitors and miglitol.

These three approaches to post-meal glucose control would work and complement one another nicely for people who have the resources to afford them. In developing countries and in the pockets of poverty in the Western world, high carb food is the norm. The vast

majority of these diabetes sufferers simply cannot afford the out-of-pocket expenses of office visits, lab tests, glucometers and medications. The one thing universally affordable for the rich and the poor alike is a timely moderate post-meal exercise. It is free, hypoglycemia is not an issue and the patient is in charge. Diabetes patients adopting this approach won't be violating any current guidelines, which encourage any-time exercise.

On the basis of elementary physiology and at least three decades of data, one initiative that could make a difference in the lives of current and future diabetes patients worldwide is to promote post-meal walks (or comparable physical activities) after the major meal of the day. The science is there. It is now up to the public and private agencies in the field and health care providers to make a concerted effort to promote timely post-meal exercise as a self-management tool for diabetes people with diabetes everywhere.

## REFERENCES

- 1 **Alouki K**, Delisle H, Besançon S, Baldé N, Sidibé-Traoré A, Drabo J, Djrolo F, Mbanya JC, Halimi S. Simple calculator to estimate the medical cost of diabetes in sub-Saharan Africa. *World J Diabetes* 2015; **6**: 1312-1322 [PMID: 26617974 DOI: 10.4239/wjcd.v6.i16.1312]
- 2 **Cavalot F**, Petrelli A, Traversa M, Bonomo K, Fiora E, Conti M, Anfossi G, Costa G and Trovati M. Postprandial blood glucose is a stronger predictor of cardiovascular events than fasting blood glucose in type 2 diabetes Mellitus, particularly in woman: lessons from the San Luigi Gonzaga Diabetes Study. *J Clin Endo Metab* 2006; **91**: 813-819 [DOI: 10.1210/jc.2005-100]
- 3 **ADA**. Standards of Medical Care in Diabetes-2013. *Diabetes Care* 2014; **Supplement 1**: S11-S66
- 4 **Handelsman Y**, Bloomgarden ZT, Grunberger G, Umpierrez G, Zimmerman RS, Bailey TS, Blonde L, Bray GA, Cohen AJ, Dagogo-Jack S, Davidson JA, Einhorn D, Ganda OP, Garber AJ, Garvey WT, Henry RR, Hirsch IB, Horton ES, Hurley DL, Jellinger PS, Jovanović L, Lebovitz HE, LeRoith D, Levy P, McGill JB, Mechanick JI, Mestman JH, Moghissi ES, Orzech EA, Pessah-Pollack R, Rosenblit PD, Vinik AI, Wyne K, Zangeneh F. American association of clinical endocrinologists and american college of endocrinology - clinical practice guidelines for developing a diabetes mellitus comprehensive care plan - 2015. *Endocr Pract* 2015; **21** Suppl 1: 1-87 [PMID: 25869408 DOI: 10.4158/EP15672.GL]
- 5 **Daenen S**, Sola-Gazagnes A, M'Bemba J, Dorange-Breillard C, Defer F, Elgrably F, Larger E, Slama G. Peak-time determination of post-meal glucose excursions in insulin-treated diabetic patients. *Diabetes Metab* 2010; **36**: 165-169 [PMID: 20226708 DOI: 10.1016/j.diabet.2009.12.002]
- 6 **Bauer LB**, Reynolds LJ, Douglas SM, Kearney ML, Hoertel HA, Shafer RS, Thyfault JP, Leidy HJ. A pilot study examining the effects of consuming a high-protein vs normal-protein breakfast on free-living glycemic control in overweight/obese 'breakfast skipping' adolescents. *Int J Obes (Lond)* 2015; **39**: 1421-1424 [PMID: 26028058 DOI: 10.1038/ijo.2015.101]
- 7 **Tay J**, Luscombe-Marsh ND, Thompson CH, Noakes M, Buckley JD, Wittert GA, Yancy WS, Brinkworth GD. A very low-carbohydrate, low-saturated fat diet for type 2 diabetes management: a randomized trial. *Diabetes Care* 2014; **37**: 2909-2918 [PMID: 25071075 DOI: 10.2337/dc14.0845]
- 8 **Nelson JD**, Poussier P, Marliss EB, Albisser AM, Zinman B. Metabolic response of normal man and insulin-infused diabetics to postprandial exercise. *Am J Physiol* 1982; **242**: E309-E316 [PMID: 7044140]
- 9 **Colberg SR**, Zarrabi L, Bennington L, Nakave A, Thomas Somma C, Swain DP, Sechrist SR. Postprandial walking is better for lowering the glycemic effect of dinner than pre-dinner exercise in type 2 diabetic individuals. *J Am Med Dir Assoc* 2009; **10**: 394-397 [PMID: 19560716 DOI: 10.1016/j.jamda.2009.03.015]
- 10 **Shin YH**, Jung HL, Ryu JW, Kim PS, Ha TY, An JY, Kang HY. Effects of a Pre-Exercise Meal on Plasma Growth Hormone Response and Fat Oxidation during Walking. *Prev Nutr Food Sci* 2013; **18**: 175-180 [PMID: 24471129 DOI: 10.3746/pnf.2013.18.3.175]
- 11 **Larsen JJ**, Dela F, Kjaer M, Galbo H. The effect of moderate exercise on postprandial glucose homeostasis in NIDDM patients. *Diabetologia* 1997; **40**: 447-453 [PMID: 9112022]
- 12 **Romijn JA**, Coyle EF, Sidossis LS, Rosenblatt J, Wolfe RR. Substrate metabolism during different exercise intensities in endurance-trained women. *J Appl Physiol* (1985) 2000; **88**: 1707-1714 [PMID: 10797133]
- 13 **Haxhi J**, Scotto di Palumbo A, Sacchetti M. Exercising for metabolic control: is timing important? *Ann Nutr Metab* 2013; **62**: 14-25 [PMID: 23208206 DOI: 10.1159/000343788]
- 14 **Derave W**, Mertens A, Muls E, Pardaens K, Hespel P. Effects of post-absorptive and postprandial exercise on glucoregulation in metabolic syndrome. *Obesity* (Silver Spring) 2007; **15**: 704-711 [PMID: 17372321]
- 15 **DiPietro L**, Gribok A, Stevens MS, Hamm LF, Rumpler W. Three 15-min bouts of moderate postmeal walking significantly improves 24-h glycemic control in older people at risk for impaired glucose tolerance. *Diabetes Care* 2013; **36**: 3262-3268 [PMID: 23761134 DOI: 10.2337/dc13-0084]
- 16 **Yamanouchi K**, Abe R, Takeda A, Atsumi Y, Shichiri M, Sato Y. The effect of walking before and after breakfast on blood glucose levels in patients with type 1 diabetes treated with intensive insulin therapy. *Diabetes Res Clin Pract* 2002; **58**: 11-18 [PMID: 12161052]
- 17 **Francois ME**, Baldi JC, Manning PJ, Lucas SJ, Hawley JA, Williams MJ, Cotter JD. 'Exercise snacks' before meals: a novel strategy to improve glycaemic control in individuals with insulin resistance. *Diabetologia* 2014; **57**: 1437-1445 [PMID: 24817675 DOI: 10.1007/s00125-014-3244-6]
- 18 **Gaudet-Savard T**, Ferland A, Broderick TL, Garneau C, Tremblay A, Nadeau A, Poirier P. Safety and magnitude of changes in blood glucose levels following exercise performed in the fasted and the postprandial state in men with type 2 diabetes. *Eur J Cardiovasc Prev Rehabil* 2007; **14**: 831-836 [PMID: 18043307]
- 19 **Poirier P**, Mawhinney S, Grondin L, Tremblay A, Broderick T, Cléroux J, Catellier C, Tancrède G, Nadeau A. Prior meal enhances the plasma glucose lowering effect of exercise in type 2 diabetes. *Med Sci Sports Exerc* 2001; **33**: 1259-1264 [PMID: 11474324]
- 20 **Poirier P**, Tremblay A, Catellier C, Tancrède G, Garneau C, Nadeau A. Impact of time interval from the last meal on glucose response to exercise in subjects with type 2 diabetes. *J Clin Endocrinol Metab* 2000; **85**: 2860-2864 [PMID: 10946894]
- 21 **Kjaer M**, Hollenbeck CB, Frey-Hewitt B, Galbo H, Haskell W, Reaven GM. Glucoregulation and hormonal responses to maximal exercise in non-insulin-dependent diabetes. *J Appl Physiol* (1985) 1990; **68**: 2067-2074 [PMID: 2193907]
- 22 **Kreisman SH**, Manzoni A, Nessim SJ, Morais JA, Gougeon R, Fisher SJ, Vranic M, Marliss EB. Glucoregulatory responses to intense exercise performed in the postprandial state. *Am J Physiol Endocrinol Metab* 2000; **278**: E786-E793 [PMID: 10780933]
- 23 **Mitchell TH**, Abraham G, Schiffrin A, Leiter LA, Marliss EB. Hyperglycemia after intense exercise in IDDM subjects during continuous subcutaneous insulin infusion. *Diabetes Care* 1988; **11**: 311-317 [PMID: 3042306]
- 24 **Yale JF**, Leiter LA, Marliss EB. Metabolic responses to intense exercise in lean and obese subjects. *J Clin Endocrinol Metab* 1989; **68**: 438-445 [PMID: 2645309]
- 25 **Heden TD**, Winn NC, Mari A, Booth FW, Rector RS, Thyfault JP, Kanaley JA. Postdinner resistance exercise improves postprandial risk factors more effectively than predinner resistance exercise in patients with type 2 diabetes. *J Appl Physiol* (1985) 2015; **118**:



- 624-634 [PMID: 25539939 DOI: 10.1152/japplphysiol.00917]
- 26 **Praet SF**, Manders RJ, Lieve AG, Kuipers H, Stehouwer CD, Keizer HA, van Loon LJ. Influence of acute exercise on hyperglycemia in insulin-treated type 2 diabetes. *Med Sci Sports Exerc* 2006; **38**: 2037-2044 [PMID: 17146308]
  - 27 **Tobin LW**, Kiens B, Galbo H. The effect of exercise on postprandial lipidemia in type 2 diabetic patients. *Eur J Appl Physiol* 2008; **102**: 361-370 [PMID: 17952452]
  - 28 **Aoi W**, Yamauchi H, Iwasa M, Mune K, Furuta K, Tanimura Y, Wada S, Higashi A. Combined light exercise after meal intake suppresses postprandial serum triglyceride. *Med Sci Sports Exerc* 2013; **45**: 245-252 [PMID: 22914246 DOI: 10.1249/MSS.0b013e31826f3107]
  - 29 **Mc Clean CM**, Mc Laughlin J, Burke G, Murphy MH, Trinick T, Duly E, Davison GW. The effect of acute aerobic exercise on pulse wave velocity and oxidative stress following postprandial hypertriglyceridemia in healthy men. *Eur J Appl Physiol* 2007; **100**: 225-234 [PMID: 17323071]
  - 30 **Cox AJ**, Pyne DB, Cox GR, Callister R, Gleeson M. Pre-exercise carbohydrate status influences carbohydrate-mediated attenuation of post-exercise cytokine responses. *Int J Sports Med* 2008; **29**: 1003-1009 [PMID: 18615388 DOI: 10.1055/s-2008-1038753]
  - 31 **Cadore EL**, Pinto RS, Bottaro M, Izquierdo M. Strength and endurance training prescription in healthy and frail elderly. *Aging Dis* 2014; **5**: 183-195 [PMID: 24900941 DOI: 10.1007/s11357-012-9405-y]

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*World Journal of Diabetes*

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Fax: +86-10-85381893

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## Daily physical activity and type 2 diabetes: A review

Hidetaka Hamasaki

Hidetaka Hamasaki, Department of Internal Medicine, National Center for Global Health and Medicine Kohnodai Hospital, Chiba 272-8516, Japan

Hidetaka Hamasaki, Hamasaki Clinic, Kagoshima 890-0046, Japan

Author contributions: Hamasaki H wrote the review.

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Correspondence to: Hidetaka Hamasaki, MD, PhD, Department of Internal Medicine, National Center for Global Health and Medicine Kohnodai Hospital, 1-7-1 Kohnodai, Chiba 272-8516, Japan. [hhamasaki78@gmail.com](mailto:hhamasaki78@gmail.com)  
 Telephone: +81-47-3723501  
 Fax: +81-47-3721858

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### Abstract

Physical activity improves glycemic control and reduces the risk of cardiovascular disease (CVD) and mortality in patients with type 2 diabetes (T2D). Moderate to vigorous physical activity is recommended to manage T2D; however, patients with T2D can be physically

weak, making it difficult to engage in the recommended levels of physical activity. Daily physical activity includes various activities performed during both occupational and leisure time such as walking, gardening, and housework that type 2 diabetic patients should be able to perform without considerable physical burden. This review focuses on the association between daily physical activity and T2D. Walking was the most common form of daily physical activity, with numerous studies demonstrating its beneficial effects on reducing the risk of T2D, CVD, and mortality. Walking for at least 30 min per day was shown to reduce the risk of T2D by approximately 50%. Additionally, walking was associated with a reduction in mortality. In contrast, evidence was extremely limited regarding other daily physical activities such as gardening and housework in patients with T2D. Recent studies have suggested daily physical activity, including non-exercise activity thermogenesis, to be favorably associated with metabolic risks and mortality. However, well-designed longitudinal studies are warranted to elucidate its effects on overall health.

**Key words:** Type 2 diabetes; Daily physical activity; Walking; Non-exercise activity thermogenesis

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**Core tip:** In addition to moderate- to vigorous-intensity exercise, daily physical activity is also important for the prevention and management of type 2 diabetes (T2D). Of note, individuals can engage in daily physical activity without remarkable physical burden, anywhere and at any time. It is well known that exercise improves the outcomes of metabolic diseases and reduces cardiovascular disease risk and mortality. However, the literature pertaining to the effects of specific types of daily physical activity on health is sparse. It is necessary to accumulate evidence on the positive effects of daily physical activity on the management of T2D.

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## INTRODUCTION

Exercise therapy is essential for the management of diabetes. A sedentary lifestyle is known to be a major risk factor of cardiovascular disease (CVD)<sup>[1]</sup>. The American College of Sports Medicine and the American Diabetes Association have recommended at least 150 min/wk of moderate (50%-70% of an individual's maximum heart rate) to vigorous (> 70% of an individual's maximum heart rate) physical activity for patients with type 2 diabetes (T2D)<sup>[2]</sup>. As for patients with type 1 diabetes, regular physical activity has also shown beneficial effects on glycemic control and other health-related outcomes, although the evidence is limited<sup>[3]</sup>.

However, the recommended intensity and duration of exercise could present a physical burden to diabetic patients and lead to cessation of exercise therapy because diabetic patients have a lower physical performance threshold than healthy individuals. Patients with T2D show a lower energy expenditure, number of steps, and duration of physical activity compared to subjects without diabetes<sup>[4]</sup>, as well as low cardiorespiratory fitness<sup>[5,6]</sup>. Moreover, the muscle strength of individuals with T2D is significantly lower than those without<sup>[7,8]</sup>. In fact, upper and lower extremity muscle strength have been shown to be inversely associated with the degree of diabetic complications<sup>[9]</sup>, suggesting that diabetes progression hinders engagement in physical activity. Indeed, the percentage of patients with diabetes found to engage in exercise therapy was approximately 40%<sup>[10]</sup>, and only 28.2% of diabetic patients in the United States achieved the recommended level of physical activity<sup>[11]</sup>. In a large-scale cohort study, individuals who performed low-volume physical activity, which was defined as 15 min/d or 90 min/wk, had a 14% reduced risk of all-cause mortality and a life expectancy increase of 3 years<sup>[12]</sup>. Thus, it is important to note that in addition to moderate- to vigorous-intensity physical activity, light- to moderate-intensity daily physical activity should also be considered an alternative and supportive exercise therapy regimen for diabetic individuals.

The purpose of this review is to highlight the effects of daily physical activity on health in type 2 diabetic patients and to further suggest a strategy for the treatment of T2D by changing the amount of daily physical activity a patient performs. This review will help physical therapists, clinicians, and patients manage T2D.

## DAILY PHYSICAL ACTIVITY

Daily physical activity is defined as continuous bodily movements via the contraction of skeletal muscle that results in an increase in energy expenditure in daily life<sup>[13]</sup>.

This includes various activities that are conducted in both occupational and leisure time such as walking, working at a desk, washing, cooking, and sports. On the other hand, exercise is defined as planned, structured, and repetitive physical activity that has the objective of improving physical fitness<sup>[13]</sup>. Physical activity is usually classified by its intensity and duration. The metabolic equivalent (MET) is a useful measurement for representing the intensity of physical activity and is defined as the amount of oxygen uptake while sitting at rest. An oxygen uptake of 3.5 mL/kg per minute is equal to the basal resting metabolic rate and is considered to be 1 MET<sup>[14]</sup>. Ainsworth *et al*<sup>[15]</sup> listed MET values for 821 specific physical activities that vary in their intensity of daily physical activity according to the situation in which they are performed. For example, walking inside is equal to only 2.0 METs (light intensity) whereas walking with children is the equivalent of 4.0 METs (moderate intensity). Therefore, it should be noted that daily physical activity covers a wide range of intensity that at times are the same as structured exercise (Table 1).

## SEDENTARY LIFESTYLE

Sedentary behavior refers to the tendency to sit during waking hours with low energy expenditures. The mean sitting time is estimated to be approximately 6-7 h/d in developed countries, and a decreased level of physical activity has been shown to be inversely associated with increased sitting time<sup>[16]</sup>. In today's world, individuals are more prone to sit on a daily basis.

Whose lifestyle is healthier: A person who engages in the recommended amount of exercise during their leisure time but is extremely sedentary in their spare time, or a person who does not achieve the recommended level of physical activity but is quite physically active in the workplace? The answer: Exercise and sedentary behavior may be mutually contradictory<sup>[17]</sup>. Sedentary time has been recognized as an independent risk factor for CVD, T2D, and all-cause mortality<sup>[17-19]</sup>. The American Diabetes Association has recommended that patients with diabetes should be encouraged to reduce their sedentary time and to not sit for more than 90 min<sup>[20]</sup>. Changing one's sedentary lifestyle to a more active lifestyle is key to the better management of T2D. In addition, sitting time may not affect all-cause mortality if it is combined with walking time, suggesting that the positive association between sitting time and mortality is only evident in individuals who sit for very long periods of time<sup>[21]</sup>. This review focuses on clinical studies that have investigated the effects of walking on CVD risk factors and mortality.

## WALKING

Walking is one of the most common physical activities of daily life<sup>[22,23]</sup>. However, 54.6% of patients with T2D have reported engaging in no weekly physical activity through walking<sup>[24]</sup>, demonstrating that patients with T2D should walk more frequently. Epidemiological studies have suggested that walking is associated

**Table 1 Excerpt of various daily physical activities and their associated metabolic equivalents**

Daily physical activity	METs
Walking	
Very slow, < 3.2 km/h	2
Slow, 3.2-4.0 km/h	2.8-3.0
For pleasure, moderate pace, 4.5-5.1 km/h	3.5
Brisk, 5.6 km/h	4.3
Very brisk, 6.4-7.2 km/h	5.0-7.0
Stair climbing, slow pace	4
Stair climbing, fast pace	8.8
Gardening	3.8
Yard work	3.0-6.0
Mowing lawn	5.5
Shoveling	5.3-7.5
Housework	
Washing dishes	1.8-2.5
Cleaning	2.3-3.8
Cooking	2.0-3.0
Child care	2.0-3.0
Elder care	2.3-4.0

Each activity is quoted from Ainsworth *et al*<sup>[15]</sup> (2011 Compendium Physical Activities). METs: Metabolic equivalents.

with a reduced risk of T2D. Hu *et al*<sup>[25]</sup> examined the relationship between physical activity, based on the use of a questionnaire, and the incidence of T2D in individuals selected from the Nurses' Health Study database. A total of 70102 women without diabetes were followed for 8 years. Overall, walking MET scores had a strong negative association with the risk of T2D. More specifically, a normal walking pace (3.2-4.8 km/h) was associated with an approximate 20%-30% reduction in the risk of T2D in women who did not engage in vigorous physical activity, and a faster walking pace was independently associated with a reduced risk of T2D. Moreover, Tanasescu *et al*<sup>[26]</sup> examined the relationship of walking with CVD risk and mortality among 3058 men with T2D. Frequent walking ( $\geq 16.1$  MET-hours/week) was associated with a nearly 40% reduced risk of mortality, and walking pace was inversely associated with CVD and total mortality independent of walking hours. Gregg *et al*<sup>[27]</sup> also investigated whether walking reduced mortality among 2896 United States adults with diabetes. Patients who walked at least 2 h/wk had a 39% lower all-cause mortality rate and a 34% lower CVD mortality rate compared with sedentary patients. However, it is important to note that these studies were observational studies, and a causal relationship between walking and incidence of T2D and/or CVD cannot therefore be deduced. Moreover, physical activity, including the amount and speed of walking, might have been over or underestimated in these studies because physical activity was evaluated by questionnaires, and such subjective data are known to have poor validity<sup>[28]</sup>, as eliminating bias is quite difficult. The Nateglinide and Valsartan in Impaired Glucose Tolerance Outcomes Research trial<sup>[29-31]</sup> investigated whether changes in ambulatory activity, assessed objectively through the use

of a pedometer, were associated with a reduced risk of cardiovascular events in individuals with impaired glucose tolerance. During a 45211 person-years follow-up, ambulatory activity was found to be inversely associated with the risk of a cardiovascular event. Specifically, an increase of 2000 steps/d at baseline was associated with a 10% reduction in having a cardiovascular event, and a 2000-step increase in daily life from baseline to 12 mo was associated with an 8% lower cardiovascular event rate<sup>[32]</sup>. On the other hand, the Nakanojo Study<sup>[33]</sup> suggested that not just walking but the combination of walking ( $> 8000$ -10000 steps/d) and physical activity at an intensity  $> 3$  METs was necessary to prevent metabolic syndrome. Daily physical activity that reaches an intensity  $> 3$  METs may need to be emphasized. A list of published articles that focused on the beneficial association of walking with the risk of T2D, CVD, and mortality is shown in Table 2.

The Diabetes Prevention Program randomly assigned 3234 individuals with impaired glucose tolerance to one of three interventions: Standard lifestyle recommendations plus metformin treatment, standard lifestyle recommendations plus placebo, or an intensive program of lifestyle modification. The lifestyle intervention reduced the incidence of diabetes by 58% after a 2.8-year follow-up. Brisk walking for at least 150 min/wk was a very effective way to prevent T2D<sup>[34]</sup>. The Da Qing IGT and Diabetes Study investigated the effects of walking for at least 30 min/d on individuals with impaired glucose tolerance. A total of 577 subjects were randomized to one of three groups: Diet only, walking only, or diet plus walking. Over a 6-year follow-up, the incidence of diabetes was significantly reduced by 46% in the walking group<sup>[35]</sup>. Kosaka *et al*<sup>[36]</sup> showed that a lifestyle intervention for men with impaired glucose tolerance effectively reduced the risk of diabetes. They recommended the following activities to their subjects to increase their physical activity: Walking for 30-40 min/d, using a staircase instead of an elevator or an escalator, performing 30 min of cycling on weekends, and getting off a bus one stop before their destination. Their findings demonstrated that the physical activity intervention combined with diet therapy reduced the risk of diabetes by 67.4%. A list of published articles with a focus on the effects of walking on the risk of T2D is shown in Table 3.

Several small-scale intervention studies have revealed favorable effects of walking on CVD risk factors. A walking program of 1 h/d for 12 wk was shown to improve physical fitness, body composition, and glycemic control in postmenopausal women with T2D<sup>[37]</sup>. Similarly, walking at least 10000 steps/d combined with diet therapy in obese patients with T2D improved their insulin sensitivity<sup>[38]</sup>. Moreover, a meta-analysis of randomized controlled trials summarized the effects of walking on glycemic control and CVD risks; specifically, walking significantly decreased glycated hemoglobin levels by 0.50% (95%CI: -0.78% to -0.21%), body mass index by -0.91 kg/m<sup>2</sup> (95%CI: -1.22 to -0.59 kg/m<sup>2</sup>),

**Table 2 Epidemiological studies investigating the associations of walking with the risk of type 2 diabetes, cardiovascular events, and mortality**

Ref.	Study design	Subjects	Physical activity measurement	Outcome, results
Hu <i>et al</i> <sup>[25]</sup>	Prospective cohort study	70102 female participants without diabetes, CVD, or cancer	MET score and walking pace based on a questionnaire	Risk of type 2 diabetes, normal walking pace (3.2-4.8 km/h): RR = 0.72; 95%CI: 0.62-0.85 Brisk or very brisk walking pace (> 4.8 km/h): RR = 0.41; 95%CI: 0.33-0.52
Tanasescu <i>et al</i> <sup>[26]</sup>	Prospective cohort study	3058 men with type 2 diabetes	MET-hour score measured by a questionnaire	Mortality, walking $\geq$ 16.1 MET-hours/week: RR = 0.60; 95%CI: 0.41-0.88 Very brisk walking pace ( $\geq$ 4 mph): RR = 0.42; 95%CI: 0.19-0.97
Gregg <i>et al</i> <sup>[27]</sup>	Prospective cohort study	2896 subjects with diabetes	Time spent walking measured by a questionnaire	Mortality, walking $\geq$ 2 h/wk, all-cause mortality: HRR = 0.61; 95%CI: 0.48-0.78; CVD mortality: HRR = 0.66; 95%CI: 0.45-0.96
Yates <i>et al</i> <sup>[32]</sup>	Prospective data analysis from the NAVIGATOR trial (a multicenter, randomized, placebo controlled, 2 $\times$ 2 factorial trial)	9306 individuals with impaired glucose tolerance	Number of steps assessed by a pedometer	Cardiovascular events, baseline ambulatory activity (2000 step/d increment): HR = 0.90; 95%CI: 0.84-0.96 Change in ambulatory activity from baseline to 12 mo (2000 step/d difference in change): HR = 0.92; 95%CI: 0.86-0.99

MET: Metabolic equivalents; RR: Relative risk; HRR: Hazard rate ratio; CVD: Cardiovascular disease; HR: Hazard ratio; NAVIGATOR: Nateglinide and Valsartan in Impaired Glucose Tolerance Outcomes Research.

**Table 3 Clinical trials investigating the effects of walking on the risk of type 2 diabetes**

Ref.	Study design	Subjects, follow-up time	Intervention	Results
Knowler <i>et al</i> <sup>[34]</sup>	Randomized clinical trial	3234 individuals with impaired glucose tolerance, 2.8 yr	A minimum of 150 min of physical activity similar intensity to brisk walking and 7% weight loss	58% reduction in the incidence rate of type 2 diabetes
Pan <i>et al</i> <sup>[35]</sup>	Randomized clinical trial	577 individuals with impaired glucose tolerance, 6 yr	At least 30 min/d of walking	46% reduction in the risk of developing diabetes
Kosaka <i>et al</i> <sup>[36]</sup>	Randomized clinical trial	458 men with impaired glucose tolerance, 4 yr	Recommendations for physical activity: walking 30-40 min/d, using staircase instead of an elevator or an escalator, 30-min cycling on weekends and getting off one bus stop before the destination	67.4% reduction in the risk of developing diabetes

and diastolic blood pressure by -1.97 mmHg (95%CI: -3.94 to -0.0 mmHg)<sup>[39]</sup>. Recently, an interesting study investigating postprandial changes in glucose, insulin, and non-esterified fatty acids in postmenopausal women at high risk of T2D was published. In this study, 22 participants were randomly assigned to one of six groups: Prolonged sitting (7.5 h) plus standing (total of 60 min), standing plus walking (total of 60 min), walking plus sitting, standing plus sitting, or walking plus standing. Both standing and walking significantly reduced postprandial glucose, insulin, and non-esterified fatty acids response compared with prolonged sitting<sup>[40]</sup>. Thus, diabetic patients may only need to stand to improve their metabolic profile.

Additionally, the way in which one walks may also be of significance. Karstoft *et al*<sup>[41]</sup> investigated the effects of interval-walking vs continuous-walking in patients with T2D. Four months of free-living interval-walking improved maximal oxygen consumption (VO<sub>2</sub> max), body composition, and glycemic control. VO<sub>2</sub> max significantly increased by 16.1%, fat mass and visceral fat significantly decreased by 10.8% and 10.6%, respectively, fasting insulin significantly decreased by 19.5%, and mean

glucose concentrations measured by the continuous glucose monitoring system significantly decreased by 8.5% in the interval-walking group, whereas no changes were observed in the continuous-walking group. Their next study focused on determining whether interval-walking improved postprandial glucose tolerance and free-living glycemic control more than continuous-walking. Their findings revealed that a greater reduction in postprandial blood glucose levels was observed with a single interval-walking session than with an oxygen consumption- and time duration-matched continuous-walking session<sup>[42]</sup>. The optimal exercise therapy for individuals with T2D and existing diabetic complications is still unknown. The amount, duration, and intensity of exercise has been emphasized in diabetes care; however, the mode of physical activity such as the variation in intensity and motion may also need to be emphasized.

## GARDENING, HOUSEWORK, AND OTHER ACTIVITIES

Gardening is the most popular daily physical activity in

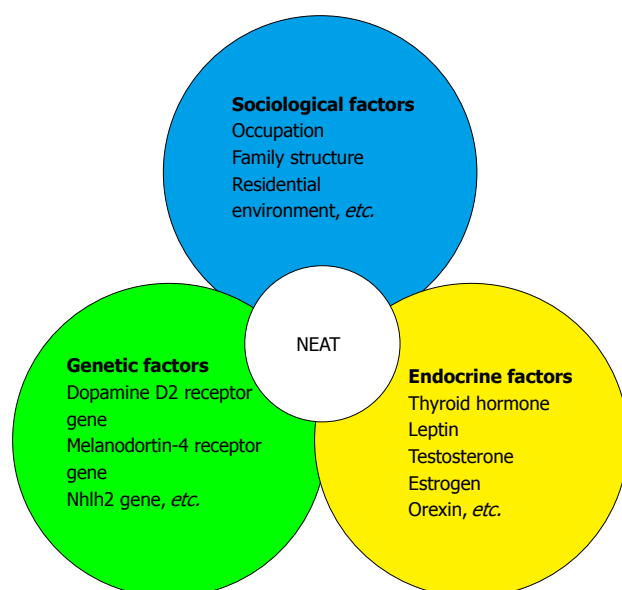
older adults<sup>[43,44]</sup>; however, the current literature does not provide sufficient evidence of the health benefits of gardening<sup>[45]</sup>. The Evaluating Long-term Diabetes Self-management Among Elder Rural Adults study, a population-based cross-sectional study, described the types of daily physical activities performed among rural older adults with diabetes. The most common physical activities reported by these individuals were walking (79.7%), housework (68.7%), and gardening (64.8%). Health-related quality of life, as measured by the short form 12-item survey scale, has been found to be positively associated with physical activity, suggesting that participating in a greater amount of daily physical activity such as gardening and housework is beneficial for older adults<sup>[46]</sup>. As domestic physical activity, which includes gardening and housework, has been analyzed as a part of total daily physical activity in previous studies, it is difficult to determine its independent effects on health. However, it is important to investigate the effects of domestic physical activity on glycemic control, CVD risk, and mortality, as domestic chores are the main contributors to total daily physical activity in patients with T2D<sup>[47]</sup>. Stamatakis *et al*<sup>[48]</sup> examined the independent effects of domestic physical activity (*e.g.*, cleaning windows, sweeping, digging, cycling, dancing) on mortality and CVD events in the Scottish Health Survey. Participation in intense domestic physical activity was associated with lower all-cause mortality (men: RR = 0.68, 95%CI: 0.50-0.91; women: RR = 0.70, 95%CI: 0.52-0.93). A recent study showed that heavy housework was associated with reduced mortality (HR = 0.71; 95%CI: 0.56-0.91) and reduced cancer deaths (HR = 0.52) in older Chinese men<sup>[49]</sup>. However, the associations between domestic physical activity and mortality or CVD risk in diabetic patients were not investigated in these studies. In contrast, Lawlor *et al*<sup>[50]</sup> found that participating in at least 2.5 h of heavy housework was not associated with an improvement in obesity among elderly British women. However, this study also lacked information concerning T2D. There is extremely little evidence to suggest that gardening and/or housework have beneficial effects on mortality, CVD risk, or other metabolic disturbances in patients with T2D. Conversely, an observational study in healthy older adults objectively measured free-living activity energy expenditure by using the doubly labeled water method and demonstrated its association with a lower risk of mortality<sup>[51]</sup>. There was an approximate 30% lower risk of mortality for every 287 kcal consumed per day by free-living activity energy expenditure; however, self-reported high-intensity exercise did not differ between the activity energy expenditure tertiles, suggesting that light- to moderate-intensity daily physical activity plays a key role in healthy living. Beddhu *et al*<sup>[52]</sup> analyzed data from the 2003-2004 National Health and Nutrition Examination Survey and showed that the duration of light-intensity physical activity (*e.g.*, casual walking, light gardening, cleaning), objectively assessed by an

accelerometer, was significantly associated with a lower mortality risk (HR = 0.59; 95%CI: 0.35-0.98) in the chronic kidney disease (CKD) population; however, an increase in moderate/vigorous activity duration did not result in a significantly lower hazard ratio in the CKD group. Approximately 20%-30% of patients with CKD had diabetes in this study, which suggests that light-intensity daily physical activity can reduce mortality in diabetic patients with CKD. Thus, these findings suggest that in contrast to moderate- to vigorous-intensity exercise, light-intensity daily physical activity may have beneficial health effects on diabetic patients who have progressed complications. Moreover, Steeves *et al*<sup>[53]</sup> conducted a study analyzing data from the 2003-2006 National Health and Nutrition Examination Survey and found that the total physical activity levels of patients with diabetes, as measured by an accelerometer, were significantly lower compared to participants with normal glucose tolerance and prediabetes. The diabetic participants were found to be inactive from 10:00 am to 8:00 pm when compared with participants with normal glucose tolerance, and their physical activity per hour declined rapidly after 12:00 pm, with the greatest difference occurring at 4:00 pm<sup>[53]</sup>. These findings suggest that clinicians should consider activity patterns in daily life as well as total physical activity to achieve optimal glycemic control in patients with T2D.

## NON-EXERCISE ACTIVITY THERMOGENESIS

Daily physical activity, with the exception of volitional sports-like activities, is defined as non-exercise activity thermogenesis (NEAT)<sup>[54]</sup>. NEAT is the main determinant of variability in total daily energy expenditure<sup>[55]</sup>, which varies substantially from person to person by up to 2000 kcal/d<sup>[56]</sup>. NEAT is influenced by various factors. For example, NEAT has been shown to increase by 25% seven days after a single bout of high-intensity walking exercise<sup>[57]</sup>. Moreover, regular exercise, especially moderate- to vigorous-intensity exercise, may increase NEAT; in contrast, living in an urban area populated with individuals who live a sedentary lifestyle will likely result in a decrease in NEAT. Indeed, the ambulation levels of rural Jamaicans was found to be more than 60% of those of urban North Americans, suggesting that urbanization is associated with a decrease in NEAT<sup>[58]</sup>. However, the intensity, frequency, and duration of physical activity required to increase NEAT is unknown. Furthermore, Levine *et al*<sup>[59]</sup> examined whether weight gain was associated with a decrease in walking distance. The consumption of an additional 1000 kcal/d significantly decreased walking distance in both lean and obese individuals, suggesting that weight gain due to overeating may result in decreased walking. Schmidt *et al*<sup>[60]</sup> examined whether 23 obesity-prone individuals and 32 obesity-resistant individuals had a different response to 3 d of overeating. They found that the walking time of





**Figure 1** Non-exercise activity thermogenesis is intricately regulated by sociological, endocrinological, and genetic factors. NEAT: Non-exercise activity thermogenesis.

obesity-prone subjects decreased significantly (-2.0%), whereas obesity-resistant subjects maintained their walking time. Taken together, these studies collectively suggest that daily diet, exercise, and living environment all influence the amount of NEAT. Furthermore, endocrine factors such as thyroid hormones<sup>[61]</sup>, leptin<sup>[62]</sup>, sex steroids<sup>[63]</sup>, and orexin<sup>[64]</sup> have all been shown to affect changes in NEAT. Several biological studies have also demonstrated that genetic variations exist in the propensity for spontaneous physical activity. For example, polymorphisms in the dopamine D2 receptor gene and melanocortin-4 receptor gene have been associated with physical activity variations in adults, and polymorphisms in the *Nhlh2* gene have shown effects on the motivation to exercise<sup>[65]</sup>. NEAT is thus modulated by endocrine, genetic, and sociological factors (Figure 1).

Previous studies have shown that NEAT plays a crucial role in the management of obesity<sup>[66]</sup>. The Look AHEAD (Action for Health in Diabetes) study did not find a reduction in the rate of cardiovascular events in obese patients with T2D after implementation of an intensive lifestyle intervention that aimed to achieve at least 7% weight loss by a reduction in dietary intake and a minimum of 175 min of moderate-intensity physical activity per week. This finding suggests that this lifestyle intervention should be conducted before an individual becomes obese<sup>[67]</sup>. Indeed, NEAT is a noteworthy factor in the management of obesity and other metabolic risks. The Shanghai Women's Health Study, a prospective cohort study, investigated the effects of exercise, walking and cycling for transportation, as well as the effect of non-exercise physical activity, on mortality. A total of 67143 women without a history of heart disease, stroke, or cancer were followed for an average of 5.7

years. That study found that women who reported 10 or more MET-hours per day of non-exercise activity had a 25%-50% lower risk of mortality compared with less active women<sup>[68]</sup>. Moreover, Hagger-Johnson *et al*<sup>[69]</sup> analyzed data from the United Kingdom Women's Cohort Study (1999-2002) to investigate the association between fidgeting behaviors and all-cause mortality. Data on sitting time and fidgeting behavior of 12778 women were analyzed. Among women in the low fidgeting group, sitting for  $\geq 7$  h/d (vs  $< 5$  h/d) was associated with a 30% increase in risk of all-cause mortality. Among women in the high fidgeting group, sitting for 5-6 h/d was associated with a decreased mortality risk (HR = 0.63, 95%CI: 0.43-0.91)<sup>[69]</sup>. Fidgeting as a component of NEAT may reduce all-cause mortality; however, little evidence is available regarding the associations between NEAT and T2D. Hamasaki *et al*<sup>[70,71]</sup> previously reported that NEAT was associated with a reduction in waist circumference, improvement in insulin sensitivity and dyslipidemia, and an increase in B-type natriuretic peptide<sup>[72]</sup> levels in patients with T2D. However, a causal relationship between NEAT and the incidence of T2D could not be deduced, as attempting to conduct a prospective study with a high level of evidence would be quite difficult because, by definition, intervention studies for NEAT would not be practical when the effect of NEAT on metabolic disease is also unknown. As physical activity is no longer an intervention for NEAT, the development of new intervention strategies to target NEAT will be an issue that should be addressed in the future.

## CONCLUSION

The current literature provides evidence of the efficacy of walking in preventing T2D and reducing the risk of cardiovascular events and/or mortality. More specifically, previous studies have suggested that brisk walking for at least 30 min/d (e.g.,  $\geq 15$  MET-hours per week) is needed to reduce the risk of T2D. Walking improves insulin sensitivity, glycemic control, and the incidence of obesity. However, there are few studies investigating the independent effects of other daily physical activities such as gardening and housework on health, especially in patients with T2D. The current literature lacks well-conducted controlled longitudinal studies investigating the effects of only daily physical activity on diabetes, dyslipidemia, hypertension, other CVD risks, and mortality. Daily physical activity, including NEAT, may be associated with a reduction in mortality. Although ensuring that patients with T2D engage in daily physical activity may be difficult, well-designed longitudinal studies that focus on daily physical activity independent of structured exercise should be conducted in the future.

## REFERENCES

- 1 Thompson PD, Buchner D, Pina IL, Balady GJ, Williams MA,

- Marcus BH, Berra K, Blair SN, Costa F, Franklin B, Fletcher GF, Gordon NF, Pate RR, Rodriguez BL, Yancey AK, Wenger NK. Exercise and physical activity in the prevention and treatment of atherosclerotic cardiovascular disease: a statement from the Council on Clinical Cardiology (Subcommittee on Exercise, Rehabilitation, and Prevention) and the Council on Nutrition, Physical Activity, and Metabolism (Subcommittee on Physical Activity). *Circulation* 2003; **107**: 3109-3116 [PMID: 12821592 DOI: 10.1161/01.CIR.0000075572.40158.77]
- 2 **Colberg SR**, Sigal RJ, Fernhall B, Regensteiner JG, Blissmer BJ, Rubin RR, Chasan-Taber L, Albright AL, Braun B; American College of Sports Medicine; American Diabetes Association. Exercise and type 2 diabetes: the American College of Sports Medicine and the American Diabetes Association: joint position statement. *Diabetes Care* 2010; **33**: e147-e167 [PMID: 21115758 DOI: 10.2337/dc10-9990]
- 3 **Yardley JE**, Hay J, Abou-Setta AM, Marks SD, McGavock J. A systematic review and meta-analysis of exercise interventions in adults with type 1 diabetes. *Diabetes Res Clin Pract* 2014; **106**: 393-400 [PMID: 25451913 DOI: 10.1016/j.diabres.2014.09.038]
- 4 **Fagour C**, Gonzalez C, Pezzino S, Florenty S, Rosette-Narece M, Gin H, Rigalleau V. Low physical activity in patients with type 2 diabetes: the role of obesity. *Diabetes Metab* 2013; **39**: 85-87 [PMID: 23159129 DOI: 10.1016/j.diabet.2012.09.003]
- 5 **Cuff DJ**, Meneilly GS, Martin A, Ignaszewski A, Tildesley HD, Frohlich JJ. Effective exercise modality to reduce insulin resistance in women with type 2 diabetes. *Diabetes Care* 2003; **26**: 2977-2982 [PMID: 14578226 DOI: 10.2337/diacare.26.11.2977]
- 6 **Ozdirenc M**, Biberoğlu S, Ozcan A. Evaluation of physical fitness in patients with Type 2 diabetes mellitus. *Diabetes Res Clin Pract* 2003; **60**: 171-176 [PMID: 12757989 DOI: 10.1016/S0168-8227(03)00064-0]
- 7 **Sayer AA**, Dennison EM, Syddall HE, Gilbody HJ, Phillips DI, Cooper C. Type 2 diabetes, muscle strength, and impaired physical function: the tip of the iceberg? *Diabetes Care* 2005; **28**: 2541-2542 [PMID: 16186295 DOI: 10.2337/diacare.28.10.2541]
- 8 **Cetinus E**, Buyukbese MA, Uzel M, Ekerbicer H, Karaoguz A. Hand grip strength in patients with type 2 diabetes mellitus. *Diabetes Res Clin Pract* 2005; **70**: 278-286 [PMID: 15878215 DOI: 10.1016/j.diabres.2005.03.028]
- 9 **Balducci S**, Sacchetti M, Orlando G, Salvi L, Pugliese L, Salerno G, D'Errico V, Iacobini C, Conti FG, Zanuso S, Nicolucci A, Pugliese G. Correlates of muscle strength in diabetes: The study on the assessment of determinants of muscle and bone strength abnormalities in diabetes (SAMBA). *Nutr Metab Cardiovasc Dis* 2014; **24**: 18-26 [PMID: 24095149 DOI: 10.1016/j.numecd.2013.04.010]
- 10 **Peyrot M**, Rubin RR, Lauritzen T, Snoek FJ, Matthews DR, Skovlund SE. Psychosocial problems and barriers to improved diabetes management: results of the Cross-National Diabetes Attitudes, Wishes and Needs (DAWN) Study. *Diabet Med* 2005; **22**: 1379-1385 [PMID: 16176200 DOI: 10.1111/j.1464-5491.2005.01644.x]
- 11 **Resnick HE**, Foster GL, Bardsley J, Ratner RE. Achievement of American Diabetes Association clinical practice recommendations among U.S. adults with diabetes, 1999-2002: the National Health and Nutrition Examination Survey. *Diabetes Care* 2006; **29**: 531-537 [PMID: 16505501 DOI: 10.2337/diacare.29.03.06.dc05-1254]
- 12 **Wen CP**, Wai JP, Tsai MK, Yang YC, Cheng TY, Lee MC, Chan HT, Tsao CK, Tsai SP, Wu X. Minimum amount of physical activity for reduced mortality and extended life expectancy: a prospective cohort study. *Lancet* 2011; **378**: 1244-1253 [PMID: 21846575 DOI: 10.1016/S0140-6736(11)60749-6]
- 13 **Caspersen CJ**, Powell KE, Christenson GM. Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research. *Public Health Rep* 1985; **100**: 126-131 [PMID: 3920711]
- 14 **Balducci S**, Sacchetti M, Haxhi J, Orlando G, D'Errico V, Fallucca S, Menini S, Pugliese G. Physical exercise as therapy for type 2 diabetes mellitus. *Diabetes Metab Res Rev* 2014; **30** Suppl 1: 13-23 [PMID: 24353273 DOI: 10.1002/dmrr.2514]
- 15 **Ainsworth BE**, Haskell WL, Herrmann SD, Meckes N, Bassett DR, Tudor-Locke C, Greer JL, Vezina J, Whitt-Glover MC, Leon AS. 2011 Compendium of Physical Activities: a second update of codes and MET values. *Med Sci Sports Exerc* 2011; **43**: 1575-1581 [PMID: 21681120 DOI: 10.1249/MSS.0b013e31821ece12]
- 16 **Bauman A**, Ainsworth BE, Sallis JF, Hagströmer M, Craig CL, Bull FC, Pratt M, Venugopal K, Chau J, Sjöström M. The descriptive epidemiology of sitting. A 20-country comparison using the International Physical Activity Questionnaire (IPAQ). *Am J Prev Med* 2011; **41**: 228-235 [PMID: 21767731 DOI: 10.1016/j.amepre.2011.05.003]
- 17 **Biswas A**, Oh PI, Faulkner GE, Bajaj RR, Silver MA, Mitchell MS, Alter DA. Sedentary time and its association with risk for disease incidence, mortality, and hospitalization in adults: a systematic review and meta-analysis. *Ann Intern Med* 2015; **162**: 123-132 [PMID: 25599350 DOI: 10.7326/M14-1651]
- 18 **Wilmot EG**, Edwardson CL, Achana FA, Davies MJ, Gorely T, Gray LJ, Khunti K, Yates T, Biddle SJ. Sedentary time in adults and the association with diabetes, cardiovascular disease and death: systematic review and meta-analysis. *Diabetologia* 2012; **55**: 2895-2905 [PMID: 22890825 DOI: 10.1007/s00125-012-2677-z]
- 19 **Hu FB**, Li TY, Colditz GA, Willett WC, Manson JE. Television watching and other sedentary behaviors in relation to risk of obesity and type 2 diabetes mellitus in women. *JAMA* 2003; **289**: 1785-1791 [PMID: 12684356 DOI: 10.1001/jama.289.14.1785]
- 20 **American Diabetes Association**. Standards of Medical Care in Diabetes-2015. *Diabetes Care* 2015; **38**: S1-S93
- 21 **Pulsford RM**, Stamatakis E, Britton AR, Brunner EJ, Hillsdon M. Associations of sitting behaviours with all-cause mortality over a 16-year follow-up: the Whitehall II study. *Int J Epidemiol* 2015; **44**: 1909-1916 [PMID: 26454871 DOI: 10.1093/ije/dyv191]
- 22 **Tanasescu M**, Leitzmann MF, Rimm EB, Willett WC, Stampfer MJ, Hu FB. Exercise type and intensity in relation to coronary heart disease in men. *JAMA* 2002; **288**: 1994-2000 [PMID: 12387651 DOI: 10.1001/jama.288.16.1994]
- 23 **Rafferty AP**, Reeves MJ, McGee HB, Pivarnik JM. Physical activity patterns among walkers and compliance with public health recommendations. *Med Sci Sports Exerc* 2002; **34**: 1255-1261 [PMID: 12165679 DOI: 10.1097/00005768-200208000-00005]
- 24 **Hays LM**, Clark DO. Correlates of physical activity in a sample of older adults with type 2 diabetes. *Diabetes Care* 1999; **22**: 706-712 [PMID: 10332670 DOI: 10.2337/diacare.22.5.706]
- 25 **Hu FB**, Sigal RJ, Rich-Edwards JW, Colditz GA, Solomon CG, Willett WC, Speizer FE, Manson JE. Walking compared with vigorous physical activity and risk of type 2 diabetes in women: a prospective study. *JAMA* 1999; **282**: 1433-1439 [PMID: 10535433 DOI: 10.1001/jama.282.15.1433]
- 26 **Tanasescu M**, Leitzmann MF, Rimm EB, Hu FB. Physical activity in relation to cardiovascular disease and total mortality among men with type 2 diabetes. *Circulation* 2003; **107**: 2435-2439 [PMID: 12719277 DOI: 10.1161/01.CIR.0000066906.11109.1F]
- 27 **Gregg EW**, Gerzoff RB, Caspersen CJ, Williamson DF, Narayan KM. Relationship of walking to mortality among US adults with diabetes. *Arch Intern Med* 2003; **163**: 1440-1447 [PMID: 12824093 DOI: 10.1001/archinte.163.12.1440]
- 28 **Shephard RJ**. Limits to the measurement of habitual physical activity by questionnaires. *Br J Sports Med* 2003; **37**: 197-206; discussion 206 [PMID: 12782543 DOI: 10.1136/bjsm.37.3.197]
- 29 **Califf RM**, Boolell M, Haffner SM, Bethel M, McMurray J, Duggal A, Holman RR; NAVIGATOR Study Group. Prevention of diabetes and cardiovascular disease in patients with impaired glucose tolerance: rationale and design of the Nateglinide And Valsartan in Impaired Glucose Tolerance Outcomes Research (NAVIGATOR) Trial. *Am Heart J* 2008; **156**: 623-632 [PMID: 18946890]
- 30 **NAVIGATOR Study Group**, Holman RR, Haffner SM, McMurray JJ, Bethel MA, Holzhauer B, Hua TA, Belenkov Y, Boolell M, Buse JB, Buckley BM, Chacra AR, Chiang FT,

- Charbonnel B, Chow CC, Davies MJ, Deedwania P, Diem P, Einhorn D, Fonseca V, Fulcher GR, Gaciong Z, Gaztambide S, Giles T, Horton E, Ilkova H, Jenssen T, Kahn SE, Krum H, Laakso M, Leiter LA, Levitt NS, Mareev V, Martinez F, Masson C, Mazzone T, Meaney E, Nesto R, Pan C, Prager R, Raptis SA, Rutten GE, Sandstroem H, Schaper F, Scheen A, Schmitz O, Sinay I, Soska V, Stender S, Tamás G, Tognoni G, Tuomilehto J, Villamil AS, Vozár J, Califf RM. Effect of nateglinide on the incidence of diabetes and cardiovascular events. *N Engl J Med* 2010; **362**: 1463-1476 [PMID: 20228402 DOI: 10.1056/NEJMoa1001122]
- 31 **NAVIGATOR Study Group**, McMurray JJ, Holman RR, Haffner SM, Bethel MA, Holzhauer B, Hua TA, Belenkov Y, Boolell M, Buse JB, Buckley BM, Chacra AR, Chiang FT, Charbonnel B, Chow CC, Davies MJ, Deedwania P, Diem P, Einhorn D, Fonseca V, Fulcher GR, Gaciong Z, Gaztambide S, Giles T, Horton E, Ilkova H, Jenssen T, Kahn SE, Krum H, Laakso M, Leiter LA, Levitt NS, Mareev V, Martinez F, Masson C, Mazzone T, Meaney E, Nesto R, Pan C, Prager R, Raptis SA, Rutten GE, Sandstroem H, Schaper F, Scheen A, Schmitz O, Sinay I, Soska V, Stender S, Tamás G, Tognoni G, Tuomilehto J, Villamil AS, Vozár J, Califf RM. Effect of valsartan on the incidence of diabetes and cardiovascular events. *N Engl J Med* 2010; **362**: 1477-1490 [PMID: 20228403 DOI: 10.1056/NEJMoa1001121]
- 32 **Yates T**, Haffner SM, Schulte PJ, Thomas L, Huffman KM, Bales CW, Califf RM, Holman RR, McMurray JJ, Bethel MA, Tuomilehto J, Davies MJ, Kraus WE. Association between change in daily ambulatory activity and cardiovascular events in people with impaired glucose tolerance (NAVIGATOR trial): a cohort analysis. *Lancet* 2014; **383**: 1059-1066 [PMID: 24361242 DOI: 10.1016/S0140-6736(13)62061-9]
- 33 **Park S**, Park H, Togo F, Watanabe E, Yasunaga A, Yoshiuchi K, Shephard RJ, Aoyagi Y. Year-long physical activity and metabolic syndrome in older Japanese adults: cross-sectional data from the Nakanajo Study. *J Gerontol A Biol Sci Med Sci* 2008; **63**: 1119-1123 [PMID: 18948564 DOI: 10.1093/gerona/63.10.1119]
- 34 **Knowler WC**, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM; Diabetes Prevention Program Research Group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 2002; **346**: 393-403 [PMID: 11832527 DOI: 10.1056/NEJMoa012512]
- 35 **Pan XR**, Li GW, Hu YH, Wang JX, Yang WY, An ZX, Hu ZX, Lin J, Xiao JZ, Cao HB, Liu PA, Jiang XG, Jiang YY, Wang JP, Zheng H, Zhang H, Bennett PH, Howard BV. Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance. The Da Qing IGT and Diabetes Study. *Diabetes Care* 1997; **20**: 537-544 [PMID: 9096977 DOI: 10.2337/diacare.20.4.537]
- 36 **Kosaka K**, Noda M, Kuzuya T. Prevention of type 2 diabetes by lifestyle intervention: a Japanese trial in IGT males. *Diabetes Res Clin Pract* 2005; **67**: 152-162 [PMID: 15649575 DOI: 10.1016/j.diabres.2004.06.010]
- 37 **Walker KZ**, Piers LS, Putt RS, Jones JA, O'Dea K. Effects of regular walking on cardiovascular risk factors and body composition in normoglycemic women and women with type 2 diabetes. *Diabetes Care* 1999; **22**: 555-561 [PMID: 10189531 DOI: 10.2337/diacare.22.4.555]
- 38 **Yamanouchi K**, Shinozaki T, Chikada K, Nishikawa T, Ito K, Shimizu S, Ozawa N, Suzuki Y, Maeno H, Kato K. Daily walking combined with diet therapy is a useful means for obese NIDDM patients not only to reduce body weight but also to improve insulin sensitivity. *Diabetes Care* 1995; **18**: 775-778 [PMID: 7555502 DOI: 10.2337/diacare.18.6.775]
- 39 **Qiu S**, Cai X, Schumann U, Velders M, Sun Z, Steinacker JM. Impact of walking on glycemic control and other cardiovascular risk factors in type 2 diabetes: a meta-analysis. *PLoS One* 2014; **9**: e109767 [PMID: 25329391 DOI: 10.1371/journal.pone.0109767]
- 40 **Henson J**, Davies MJ, Bodicoat DH, Edwardson CL, Gill JM, Stensel DJ, Tolfrey K, Dunstan DW, Khunti K, Yates T. Breaking Up Prolonged Sitting With Standing or Walking Attenuates the Postprandial Metabolic Response in Postmenopausal Women: A Randomized Acute Study. *Diabetes Care* 2016; **39**: 130-138 [PMID: 26628415 DOI: 10.2337/dc15-1240]
- 41 **Karstoft K**, Winding K, Knudsen SH, Nielsen JS, Thomsen C, Pedersen BK, Solomon TP. The effects of free-living interval-walking training on glycemic control, body composition, and physical fitness in type 2 diabetic patients: a randomized, controlled trial. *Diabetes Care* 2013; **36**: 228-236 [PMID: 23002086 DOI: 10.2337/dc12-0658]
- 42 **Karstoft K**, Christensen CS, Pedersen BK, Solomon TP. The acute effects of interval- Vs continuous-walking exercise on glycemic control in subjects with type 2 diabetes: a crossover, controlled study. *J Clin Endocrinol Metab* 2014; **99**: 3334-3342 [PMID: 24905068 DOI: 10.1210/jc.2014-1837]
- 43 **Rowinski R**, Dabrowski A, Kostka T. Gardening as the dominant leisure time physical activity (LTPA) of older adults from a post-communist country. The results of the population-based PolSenior Project from Poland. *Arch Gerontol Geriatr* 2015; **60**: 486-491 [PMID: 25661458 DOI: 10.1016/j.archger.2015.01.011]
- 44 **Mooney SJ**, Joshi S, Cerdá M, Quinn JW, Beard JR, Kennedy GJ, Benjamin EO, Ompad DC, Rundle AG. Patterns of Physical Activity Among Older Adults in New York City: A Latent Class Approach. *Am J Prev Med* 2015; **49**: e13-e22 [PMID: 26091927 DOI: 10.1016/j.amepre.2015.02.015]
- 45 **Nicklellt EJ**, Anderson LA, Yen IH. Gardening Activities and Physical Health Among Older Adults: A Review of the Evidence. *J Appl Gerontol* 2014 Dec 16; Epub ahead of print [PMID: 25515757 DOI: 10.1177/0733464814563608]
- 46 **Arcury TA**, Snively BM, Bell RA, Smith SL, Stafford JM, Wetmore-Arkader LK, Quandt SA. Physical activity among rural older adults with diabetes. *J Rural Health* 2006; **22**: 164-168 [PMID: 16606429 DOI: 10.1111/j.1748-0361.2006.00026.x]
- 47 **Cloix L**, Caille A, Helmer C, Bourdel-Marchasson I, Fagot-Campagna A, Fournier C, Lecomte P, Oppert JM, Jacobi D. Physical activity at home, at leisure, during transportation and at work in French adults with type 2 diabetes: the ENTRED physical activity study. *Diabetes Metab* 2015; **41**: 37-44 [PMID: 25277526 DOI: 10.1016/j.diabet.2014.07.003]
- 48 **Stamatakis E**, Hamer M, Lawlor DA. Physical activity, mortality, and cardiovascular disease: is domestic physical activity beneficial? The Scottish Health Survey -- 1995, 1998, and 2003. *Am J Epidemiol* 2009; **169**: 1191-1200 [PMID: 19329529 DOI: 10.1093/aje/kwp042]
- 49 **Yu R**, Leung J, Woo J. Housework reduces all-cause and cancer mortality in Chinese men. *PLoS One* 2013; **8**: e61529 [PMID: 23667441 DOI: 10.1371/journal.pone.0061529]
- 50 **Lawlor DA**, Taylor M, Bedford C, Ebrahim S. Is housework good for health? Levels of physical activity and factors associated with activity in elderly women. Results from the British Women's Heart and Health Study. *J Epidemiol Community Health* 2002; **56**: 473-478 [PMID: 12011209 DOI: 10.1136/jech.56.6.473]
- 51 **Manini TM**, Everhart JE, Patel KV, Schoeller DA, Colbert LH, Visser M, Tylavsky F, Bauer DC, Goodpaster BH, Harris TB. Daily activity energy expenditure and mortality among older adults. *JAMA* 2006; **296**: 171-179 [PMID: 16835422 DOI: 10.1001/jama.296.2.171]
- 52 **Beddhu S**, Wei G, Marcus RL, Chonchol M, Greene T. Light-intensity physical activities and mortality in the United States general population and CKD subpopulation. *Clin J Am Soc Nephrol* 2015; **10**: 1145-1153 [PMID: 25931456 DOI: 10.2215/CJN.08410814]
- 53 **Steeves JA**, Murphy RA, Crainiceanu CM, Zipunnikov V, Van Domelen DR, Harris TB. Daily Patterns of Physical Activity by Type 2 Diabetes Definition: Comparing Diabetes, Prediabetes, and Participants with Normal Glucose Levels in NHANES 2003-2006. *Prev Med Rep* 2015; **2**: 152-157 [PMID: 25909051 DOI: 10.1016/j.pmedr.2015.02.007]
- 54 **Levine JA**. Non-exercise activity thermogenesis (NEAT). *Nutr Rev* 2004; **62**: S82-S97 [PMID: 15387473 DOI: 10.1111/j.1753-4887.2004.tb00094.x]
- 55 **Donahoo WT**, Levine JA, Melanson EL. Variability in energy expenditure and its components. *Curr Opin Clin Nutr Metab Care*

- 2004; **7**: 599-605 [PMID: 15534426 DOI: 10.1097/00075197-200411000-00003]
- 56 **Levine JA**, Vander Weg MW, Hill JO, Klesges RC. Non-exercise activity thermogenesis: the crouching tiger hidden dragon of societal weight gain. *Arterioscler Thromb Vasc Biol* 2006; **26**: 729-736 [PMID: 16439708 DOI: 10.1161/01.ATV.0000205848.83210.73]
- 57 **Alahmadi MA**, Hills AP, King NA, Byrne NM. Exercise intensity influences nonexercise activity thermogenesis in overweight and obese adults. *Med Sci Sports Exerc* 2011; **43**: 624-631 [PMID: 21412111 DOI: 10.1249/MSS.0b013e3181f7a0cb]
- 58 **Levine JA**, McCrady SK, Boyne S, Smith J, Cargill K, Forrester T. Non-exercise physical activity in agricultural and urban people. *Urban Stud* 2011; **48**: 2417-2427 [PMID: 22073428 DOI: 10.1177/0042098010379273]
- 59 **Levine JA**, McCrady SK, Lanningham-Foster LM, Kane PH, Foster RC, Manohar CU. The role of free-living daily walking in human weight gain and obesity. *Diabetes* 2008; **57**: 548-554 [PMID: 18003759 DOI: 10.2337/db07-0815]
- 60 **Schmidt SL**, Harmon KA, Sharp TA, Kealey EH, Bessesen DH. The effects of overfeeding on spontaneous physical activity in obesity prone and obesity resistant humans. *Obesity* (Silver Spring) 2012; **20**: 2186-2193 [PMID: 22522883 DOI: 10.1038/oby.2012.103]
- 61 **Levine JA**, Nygren J, Short KR, Nair KS. Effect of hyperthyroidism on spontaneous physical activity and energy expenditure in rats. *J Appl Physiol* (1985) 2003; **94**: 165-170 [PMID: 12486020]
- 62 **Levine JA**, Eberhardt NL, Jensen MD. Leptin responses to overfeeding: relationship with body fat and nonexercise activity thermogenesis. *J Clin Endocrinol Metab* 1999; **84**: 2751-2754 [PMID: 10443673 DOI: 10.1210/jc.84.8.2751]
- 63 **Bowen RS**, Knab AM, Hamilton AT, McCall JR, Moore-Harrison TL, Lightfoot JT. Effects of Supraphysiological Doses of Sex Steroids on Wheel Running Activity in Mice. *J Steroids Horm Sci* 2012; **3**: 110 [PMID: 25419484 DOI: 10.4172/2157-7536.1000110]
- 64 **Kotz CM**. Integration of feeding and spontaneous physical activity: role for orexin. *Physiol Behav* 2006; **88**: 294-301 [PMID: 16787655]
- 65 **Garland T**, Schutz H, Chappell MA, Keeney BK, Meek TH, Copes LE, Acosta W, Drenowatz C, Maciel RC, van Dijk G, Kotz CM, Eisenmann JC. The biological control of voluntary exercise, spontaneous physical activity and daily energy expenditure in relation to obesity: human and rodent perspectives. *J Exp Biol* 2011; **214**: 206-229 [PMID: 21177942 DOI: 10.1242/jeb.048397]
- 66 **Villablanca PA**, Alegria JR, Mookadam F, Holmes DR, Wright RS, Levine JA. Nonexercise activity thermogenesis in obesity management. *Mayo Clin Proc* 2015; **90**: 509-519 [PMID: 25841254 DOI: 10.1016/j.mayocp.2015.02.001]
- 67 **Look AHEAD Research Group**, Wing RR, Bolin P, Brancati FL, Bray GA, Clark JM, Coday M, Crow RS, Curtis JM, Egan CM, Espeland MA, Evans M, Foreyt JP, Ghazarian S, Gregg EW, Harrison B, Hazuda HP, Hill JO, Horton ES, Hubbard VS, Jakicic JM, Jeffery RW, Johnson KC, Kahn SE, Kitabchi AE, Knowler WC, Lewis CE, Maschak-Carey BJ, Montez MG, Murillo A, Nathan DM, Patricio J, Peters A, Pi-Sunyer X, Pownall H, Reboussin D, Regensteiner JG, Rickman AD, Ryan DH, Safford M, Wadden TA, Wagenknecht LE, West DS, Williamson DF, Yanovski SZ. Cardiovascular effects of intensive lifestyle intervention in type 2 diabetes. *N Engl J Med* 2013; **369**: 145-154 [PMID: 23796131 DOI: 10.1056/NEJMoa1212914]
- 68 **Matthews CE**, Jurj AL, Shu XO, Li HL, Yang G, Li Q, Gao YT, Zheng W. Influence of exercise, walking, cycling, and overall nonexercise physical activity on mortality in Chinese women. *Am J Epidemiol* 2007; **165**: 1343-1350 [PMID: 17478434]
- 69 **Hagger-Johnson G**, Gow AJ, Burley V, Greenwood D, Cade JE. Sitting Time, Fidgeting, and All-Cause Mortality in the UK Women's Cohort Study. *Am J Prev Med* 2016; **50**: 154-160 [PMID: 26416340 DOI: 10.1016/j.amepre.2015.06.025]
- 70 **Hamasaki H**, Yanai H, Mishima S, Mineyama T, Yamamoto-Honda R, Kakei M, Ezaki O, Noda M. Correlations of non-exercise activity thermogenesis to metabolic parameters in Japanese patients with type 2 diabetes. *Diabetol Metab Syndr* 2013; **5**: 26 [PMID: 23711224 DOI: 10.1186/1758-5996-5-26]
- 71 **Hamasaki H**, Noda M, Moriyama S, Yoshikawa R, Katsuyama H, Sako A, Mishima S, Kakei M, Ezaki O, Yanai H. Daily Physical Activity Assessed by a Triaxial Accelerometer Is Beneficially Associated with Waist Circumference, Serum Triglycerides, and Insulin Resistance in Japanese Patients with Prediabetes or Untreated Early Type 2 Diabetes. *J Diabetes Res* 2015; **2015**: 526201 [PMID: 26064983 DOI: 10.1155/2015/526201]
- 72 **Hamasaki H**, Yanai H, Kakei M, Noda M, Ezaki O. The association between daily physical activity and plasma B-type natriuretic peptide in patients with glucose intolerance: a cross-sectional study. *BMJ Open* 2015; **5**: e006276 [PMID: 25596197 DOI: 10.1136/bmjopen-2014-006276]

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Retrospective Cohort Study

## Does parity worsen diabetes-related chronic complications in women with type 1 diabetes?

Marilia Brito Gomes, Carlos Antonio Negrato, Ana Almeida, Antonio Ponce de Leon

Marilia Brito Gomes, Ana Almeida, Department of Internal Medicine, Diabetes Unit, State University of Rio de Janeiro, Rio de Janeiro 20550-900, Brazil

Carlos Antonio Negrato, Bauru's Diabetics Association, Bauru, São Paulo 17012-433, Brazil

Antonio Ponce de Leon, Department of Epidemiology, State University of Rio de Janeiro, Rio de Janeiro 20550-900, Brazil

**Author contributions:** Gomes MB, Negrato CA, Almeida A and de Leon AP analyzed the data and wrote the manuscript; Almeida A collected the data.

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**Correspondence to:** Carlos Antonio Negrato, MD, PhD, Bauru's Diabetics Association, Rua Saint Martin 27-07, Bauru, São Paulo 17012-433, Brazil. [carlosnegrato@uol.com.br](mailto:carlosnegrato@uol.com.br)  
 Telephone: +55-14-32348915  
 Fax: +55-14-32348915

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### Abstract

**AIM:** To determine the relationship between parity, glycemic control, cardiovascular risk factors and diabetes-related chronic complications in women with type 1 diabetes.

**METHODS:** This was a multicenter cross-sectional study conducted between December 2008 and December 2010 in 28 public clinics in 20 cities from the 4 Brazilian geographic regions. Data were obtained from 1532 female patients, 59.2% Caucasians, and aged  $25.2 \pm 10.6$  years. Diabetes duration was of  $11.5 \pm 8.2$  years. Patient's information was obtained through a questionnaire and a chart review. Parity was stratified in five groups: Group 0 (nulliparous), group 1 (1 pregnancy), group 2 (2 pregnancies), group 3 (3 pregnancies), group 4 ( $\geq 4$  pregnancies). Test for trend and multivariate random intercept logistic and linear regression models were used to evaluate the effect of parity upon glycemic control, cardiovascular risk factors and diabetes-related complications.

**RESULTS:** Parity was not related with glycemic control and nephropathy. Moreover, the effect of parity upon hypertension, retinopathy and macrovascular disease did not persist after adjustments for demographic and

clinical variables in multivariate analysis. For retinopathy, the duration of diabetes and hypertension were the most important independent variables and for macrovascular disease, these variables were age and hypertension. Overweight or obesity was noted in a total of 538 patients (35.1%). A linear association was found between the frequency of overweight or obesity and parity ( $P = 0.004$ ). Using a random intercept multivariate linear regression model with body mass index (BMI) as dependent variable a borderline effect for parity ( $P = 0.06$ ) was noted after adjustment for clinical and demographic data. The observed variability of BMI was not attributable to differences between centers.

**CONCLUSION:** Our results suggest that parity has a borderline effect on body mass index but does not have an important effect upon hypertension and micro or macrovascular chronic complications. Future prospective evaluations must be conducted to clarify the relationship between parity, appearance or worsening of diabetes-related chronic complications.

**Key words:** Type 1 diabetes; Parity; Glycemic control; Cardiovascular risk factors; Diabetes-related chronic complications

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**Core tip:** To the best of our knowledge, this was the largest study ever conducted with pregnant women with type 1 diabetes in Brazil and maybe in Latin America. Our results suggest that parity did not have an important effect upon hypertension and micro or macrovascular diabetes-related chronic complications. Further prospective studies with a larger number of patients must be addressed to clarify the relationship between parity, appearance or worsening of diabetes-related chronic complications.

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## INTRODUCTION

There is a controversy about the impact of pregnancy and parity on the appearance of diabetes-related chronic complications or the progression of its course if they are already present in women with preexisting type 1 diabetes (T1D)<sup>[1,2]</sup>.

Some studies found a worsening of retinopathy during pregnancy<sup>[3-5]</sup>, which was not confirmed by others<sup>[2-4,6]</sup>. The worsening of retinopathy could be explained by several risk factors such as pregnancy *per se*, hypertension, hyperglycemia, duration of diabetes and a rapid drop in blood glucose levels aiming to reach normoglycemia<sup>[5]</sup>.

Also the presence of increased circulating levels of insulin-like growth factor (IGF-1) that occurs normally during pregnancy could accelerate the progression of an already existing retinopathy<sup>[7]</sup>. The association between pregnancy and nephropathy is related to an increased albuminuria or alterations on glomerular filtration rate<sup>[8]</sup>. So far, the mechanisms linking pregnancy to both chronic complications are still unclear and controversial. Some studies showed an association between improvement of glycemic control under intensive insulin therapy and worsening of retinopathy but not nephropathy<sup>[5,9]</sup>.

Other conditions involved in the pathophysiology of diabetes-related chronic complications must be addressed such as pre-pregnancy body mass index (BMI) and blood pressure levels, which have been increasing in the last three decades in some populations<sup>[10]</sup>. In a Swedish study, it was found that the combination of T1D and overweight/obesity confers a high risk for adverse outcomes, like pre-eclampsia, that increases proportionally to BMI<sup>[11]</sup>. Otherwise, when women with T1D presenting the features of metabolic syndrome become pregnant, they generally have the coexistence of vascular complications<sup>[12]</sup>. It has also been shown that women with T1D and pre-eclampsia or pregnancy-induced hypertension present high risk of severe retinopathy later in life<sup>[13]</sup>.

In the Eurodiab study a better glycemic control was found among parous women than nulliparous, and parity did not influence the levels of microalbuminuria and preexisting retinopathy<sup>[14]</sup>. In a Finnish study it was found a slower progression of retinopathy in parous women than in nulliparous<sup>[15]</sup>.

Considering the scarcity of data regarding the relationship between parity, glycemic control and diabetes-related chronic complications in women with T1D in Brazil, the Brazilian Type 1 Diabetes Study Group (BrazDiab1SG) conducted this survey aiming to analyze the impact of parity in the above mentioned clinical conditions.

## MATERIALS AND METHODS

### Patients and methods

This was a multicenter, cross-sectional, observational study conducted between December 2008 and December 2010 in 28 public secondary and tertiary care-level clinics from the National Brazilian Health Care System, located in 20 cities in all Brazilian geographic regions (North/Northeast, Mid-West, Southeast, and South). The details of the data collection methods have been published previously<sup>[16]</sup>. Two thousand and ten patients, 56% female (2010, 56% female) patients that were diagnosed between 1960 and 2010 were included in the study. Among the 2010 enrolled women, only those who knew their age at menarche were included ( $n = 1532$ , 76.2%). Patients who did not have had menarche ( $n = 467$ , 23.2%) and women with incomplete information for parity ( $n = 11$ , 0.5%) were excluded.

Each local ethics committees approved the study (Appendix 1). All patients or their parents, when necessary, signed a written informed consent agreeing

with their participation in the study.

During a clinical visit, a questionnaire was applied in order to collect demographic, educational and economic data. The following variables were assessed then: Age, age at diagnosis, duration of diabetes, height (m), weight (kg), blood pressure, parity, comorbidities, smoking status and the use of metformin.

Data from the last clinical visit were obtained from medical records such as levels of glycated hemoglobin (HbA1c), fasting plasma glucose (FPG), total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides. Diabetes-related chronic complications were screened in all patients with diabetes duration longer than 5 years, such as retinopathy (by fundoscopy; classified as absent, non-proliferative or proliferative), clinical nephropathy [according to American Diabetes Association (ADA)] recommendations<sup>[17]</sup>, macrovascular diseases (clinical coronary artery disease, stroke, and peripheral vascular disease), and foot alterations. The following goals for adequate metabolic control that are adopted by the ADA<sup>[17]</sup> were also adopted by the BrazDiab1SG: HbA1c at goal was defined as HbA1c levels of < 58 mmol/mol (7.5%) for patients with T1D between 13 and 19 years old; < 64 mmol/mol (8%) for patients between 6 and 12 years old; between 58 mmol/mol (7.5%) and 69 mmol/mol (8.5%) for patients < 6 years old; and < 53 mmol/mol (7%) for adult patients<sup>[17]</sup>. Poor glycemic control was considered as having HbA1c levels higher than 75 mmol/mol (9%).

Hypertension was defined as a systolic blood pressure (sBP)  $\geq 140$  mmHg and/or diastolic blood pressure (dBP)  $\geq 90$  mmHg, use of antihypertensive agents or self-reported for adults; in adolescents hypertension was defined as a sBP or dBP  $\geq 95^{\text{th}}$  percentile for age, sex and height<sup>[17]</sup>.

Overweight was defined as a BMI  $\geq 25$  kg/m<sup>2</sup>, and obesity as a BMI  $\geq 30$  kg/m<sup>2</sup> in adults<sup>[18]</sup>. Overweight was considered as a BMI of  $\geq 85^{\text{th}}$  percentile for age and gender, and obesity as a BMI of  $\geq 95^{\text{th}}$  percentile for age and gender for adolescents<sup>[18]</sup>.

In 1347 patients (88.0%), HbA1c was measured using methods certified by the National Glycohemoglobin Standardization Program (NGSP): High-performance liquid chromatography in 733 patients (54.3%) and turbidimetry in 614 patients (45.7%). Measurement of HbA1c levels using methods that were not certified by the NGSP and patients with no data on HbA1c levels or use of methodology not certified by the NGSP were not included in the analyses of glycemic control ( $n = 185$ , 12.0%). Enzymatic techniques were used to measure FPG, triglycerides, HDL cholesterol, and total cholesterol. Friedewald's equation was used to calculate LDL cholesterol<sup>[19]</sup>. Patients smoking more than one cigarette per day at the time of the interview were considered as current smokers.

### Sample calculation and economic status

The study sample calculation was done according to a methodology described elsewhere<sup>[16]</sup>. Our sample represented the distribution of T1D cases all over Brazil that was estimated using the overall population distribution reported in the 2000 Brazilian Institute of Geography and Statistics Population Census (IBGE)<sup>[20]</sup>. These data were combined with national estimates of diabetes prevalence determined by a survey conducted in 1988 in order to determine the minimum number of patients that should be studied in each geographic region of the country<sup>[21]</sup>. Economic status was defined according to the Brazilian Economic Classification Criteria<sup>[22]</sup>. This classification also takes in account the education level: Illiterate/incomplete primary education, complete primary education/incomplete secondary education, complete secondary education/incomplete high school, complete high school/some college or college graduate. The following economic status categories were considered: High, middle, low, and very low<sup>[22]</sup>.

### Statistical analysis

The data were summarized as means ( $\pm$  SD) and median (minimum-maximum) for continuous variables and as counts (relative frequencies) for discrete variables. Patients were stratified in five groups according to parity: Group 0 (nulliparous), group 1 (1 pregnancy), group 2 (2 pregnancies), group 3 (3 pregnancies) and group 4 ( $\geq 4$  pregnancies).

ANOVA test with Sidak correction was used. Test for trend (linear association) was used to analyze the association between parity and frequency of retinopathy, albuminuria and hypertension. A multivariate random intercept logistic regression model was performed with retinopathy (yes/no) as the dependent (outcome) variable and parity as the independent (exposure) variable. Other independent variables, such as socioeconomic status, ethnicity (Caucasian or non-Caucasian based on self-reporting), age, duration of diabetes, HbA1c levels and hypertension (yes/no) were also controlled in the analysis. The same multivariate model was performed with the following dependent variables: Hypertension (yes/no), adding to the set of independent variables, creatinine levels, BMI and smoking status and excluding hypertension; macrovascular disease with the same demographic variables above-mentioned as independent variables adding to the model: Hypertension (yes/no), HbA1c and LDL-Cholesterol levels and smoking status (yes/no). A random intercept multivariate linear regression model was further applied to BMI as dependent variable (three nested models were considered). All analyses were performed using the SPSS version 17.0, SPSS, Inc., Chicago, Illinois, United States, except the random intercept models that were fitted using MLwiN<sup>[23]</sup>. Odds ratios (ORs) with 95% CIs, variance and standard error

**Table 1 Clinical and demographic data of the studied population**

Variable	
<i>n</i>	1532
Age, yr	25.2 ± 10.6
Age at diabetes diagnosis, yr	11.4 ± 8.1
Age at menarche, yr	12.7 ± 1.7
Duration of diabetes, yr	11.5 ± 8.2
Ethnicity, <i>n</i> (%) <sup>1</sup>	
Caucasian	907 (59.2)
Non-caucasian <sup>1</sup>	625 (40.7)
Geographic region, <i>n</i> (%)	
Southeast	611 (39.9)
North/Northeast	454 (29.6)
South	367 (24.0)
Mid-west	100 (6.5)
Economic status	
High	104 (6.7)
Medium	383 (25.0)
Low	533 (34.8)
Very low	512 (33.4)
Level of care, <i>n</i> (%)	
Secondary	412 (26.9)
Tertiary	1117 (73.1)
Time of follow-up, yr	7.1 (< 1 to 49)

Data are presented as number (percentage), mean ± SD or median (minimum/maximum). <sup>1</sup>African-Brazilians, Mulattos, Asians, and Native Indians.

were calculated when indicated. A two-sided *P* value less than 0.05 was considered significant.

The statistical review of the study was performed by a biomedical statistician that is also a co-author (APL).

## RESULTS

### Overview of the studied population

Data were obtained from 1532 patients (excluded *n* = 478, 23.7%). The economic status of 1045 (68.2%) of the patients was either very low or low. Table 1 lists the demographic data of the studied population.

### Overview of the studied population stratified according to parity, demographics and socioeconomic status data

The comparison between the patients stratified according to parity showed that patients from groups 0, 1 and 2 were younger than patients from group 4 (*P* < 0.001). Patients from groups 0 and 1 had been diagnosed with diabetes with lower age and had less duration of diabetes than patients from the other groups (*P* < 0.001). A difference between the five groups and geographic regions of the country was observed, being the difference accounted by Mid-West region, that had no patients in group four. These data are described in Table 2.

### Overview of the studied population stratified according to parity, glycemic and cardiovascular risk factors control

Overweight or obesity was noted in 538 patients (35.1%). Patients from group 0 had lower BMI than patients from the other groups. A linear association was found between

the frequency of overweight or obesity and parity (*P* = 0.004). Using a random intercept multivariate linear regression model with BMI as dependent variable a borderline effect for parity (*P* = 0.06) was noted after adjustment for clinical and demographic data (model 2 and model 3). The significant effect of low insulin dose and age persisted. The observed variability of BMI was not attributable to centers. These data are described in Table 3.

A lower level of HbA1c was found in patients from group 2 in comparison to patients from group 0. No differences between the five groups were observed for the number of patients reaching the target of HbA1c. A higher frequency of hypertension and higher levels of SBP and DBP were observed in group 4 in comparison to the other groups (*P* < 0.01 for all comparisons).

A higher HDL-cholesterol was observed in group 4 in comparison to the other groups. No other difference in lipid parameters was noted. Metformin was used by 162 (10.6 %) patients, and its use was related to parity (*P* = 0.02). The use of metformin was more frequently found in patients from group 4 in comparison with patients from groups 0, 1 and 3, respectively 9 (17.3%) vs 103 (10.2%) vs 20 (8.4) vs 7 (7.4), *P* = 0.04. A higher insulin dose/kg was used by patients from group 0 in comparison to patients from the other groups. The demographic, clinical, and laboratory data of patients stratified by parity are described in Table 2.

### Overview of the studied population stratified according to parity and diabetes-related chronic complications

Overall, 1219 (79.7%) of the patients had criteria to be screened for diabetes-related chronic complications. Parity was related to the presence of diabetes-related chronic complications both micro and macrovascular. Considering women with information regarding retinopathy, (*n* = 1033, 84.7%) a lower frequency of non-proliferative and proliferative retinopathy was noted in patients from group 0 in comparison to the other groups (*P* < 0.01). A tendency for an association between parity and nephropathy was observed (*P* = 0.08) in those patients with information obtained in the previous year (*n* = 1041, 85.4%). These data are shown in Table 2.

Using a multivariate random intercept logistic regression model with retinopathy as the dependent variable no effect of parity was noted but the OR for duration of diabetes and presence of hypertension were 1.11 (95%CI: 1.08-1.14, *P* < 0.001) and 3.51 (95%CI: 2.42-5.08, *P* < 0.001), respectively. The other independent variables did not reach statistical significance. The same model with macrovascular disease as dependent variable also showed no effect of parity but the OR for age was 1.067 (95%CI: 1.03-1.106, *P* < 0.0001), while for HbA1c levels it was 1.166 (95%CI: 1.023-1.330, *P* < 0.02) and for hypertension it was 2.29 (95%CI: 1.219-4.306, *P* < 0.02). The other independent variables did not reach statistical significance.

In multivariate random intercept logistic regression



**Table 2** Clinical, demographic and laboratory data stratified by parity

Variable	Parity					<sup>1</sup> P-value
	Group 0 (nulliparous)	Group 1 (1 pregnancy)	Group 2 (2 pregnancies)	Group 3 (3 pregnancies)	Group 4 (≥ 4 pregnancies)	
<i>n</i> (%)	1014 (66.2)	238 (15.5)	147 (9.6)	81 (5.3)	52 (3.4)	
Demographic and economic status data						
Age, yr	20.7 ± 7.9	30.2 ± 8.5	34.6 ± 8.6	38.4 ± 9.6	42.3 ± 10.7	< 0.001
< 15	191 (18.7)	1 (0.4)	0	0	0	
15-30	706 (69.6)	117 (49.2)	42 (28.6)	12 (14.8)	6 (11.5)	
≥ 30	117 (11.5)	120 (50.4)	105 (71.4)	69 (85.2)	46 (88.5)	
Duration of DM, yr	9.4 ± 6.9	13.9 ± 8.2	15.3 ± 8.9	17.9 ± 8.9	19.0 ± 10.7	< 0.001
Age at diagnosis, yr	11.2 ± 6.4	16.2 ± 8.4	19.2 ± 8.1	20.5 ± 9.3	23.3 ± 8.8	< 0.001
Ethnicity, yr (%) <sup>2</sup>						0.7
Caucasian	606 (59.8)	144 (60.5)	83 (56.5)	47 (58.0)	27 (51.9)	
Non-caucasian	408 (40.2)	94 (39.5)	64 (43.5)	34 (42.0)	25 (48.1)	
Geographic region, <i>n</i>						0.001
Southeast	410 (40.4)	94 (39.5)	54 (36.7)	31 (38.3)	22 (42.3)	
South	248 (24.5)	53 (22.3)	36 (24.5)	18 (22.2)	12 (23.1)	
North/Northeast	309 (30.5)	69 (29.0)	39 (26.5)	19 (23.5)	18 (34.6)	
Mid-West	47 (4.6)	22 (9.2)	18 (12.2)	13 (16.0)	0	
Economic status (%)						0.5
High	72 (7.1)	16 (6.7)	8 (5.4)	5 (6.2)	3 (5.8)	
Medium	273 (26.9)	56 (23.5)	29 (19.7)	18 (22.2)	7 (13.5)	
Low	340 (33.5)	85 (35.7)	55 (37.4)	33 (40.7)	20 (38.5)	
Very low	329 (32.4)	81 (34.0)	55 (37.4)	25 (30.9)	22 (42.3)	
Glycemic control and insulin dose						
HbA1c (%)	9.6 ± 2.6	9.4 ± 2.4	8.8 ± 2.0	9.4 ± 2.4	9.5 ± 2.0	0.02
HbA1c (mmol/mol)	81.9 ± 28.4	79.9 ± 26.3	73.4 ± 22.2	79.5 ± 26.8	80.4 ± 21.7	
HbA1c (good) <i>n</i> (%)	107 (11.9)	21 (10.3)	19 (14.6)	8 (11.3)	3 (6.5)	0.1
H1Ac (poor) <i>n</i> (%)	480 (53.5)	99 (48.8)	51 (39.2)	37 (52.1)	27 (58.7)	
Insulin dose (U/kg per day)	0.98 ± 0.4	0.8 ± 0.3	0.8 ± 0.3	0.7 ± 0.4	0.8 ± 0.4	0.001
Metformin use, yr (%)	103 (10.2)	20 (8.4)	24 (16.3)	6 (7.4)	9 (17.3)	0.04
Cardiovascular risk factors						
sBP (mmHg)	110.8 ± 14.6	117.5 ± 15.7	119.6 ± 18.7	119.8 ± 18.9	124.0 ± 21.4	< 0.001
dBp (mmHg)	72.3 ± 10.1	75.2 ± 11.3	74.9 ± 11.5	75.9 ± 11.5	75.9 ± 10.2	< 0.001
Hypertension, yr (%)	158 (16.6)	76 (32.2)	53 (36.3)	29 (35.8)	25 (48.1)	< 0.001
Cholesterol (mg/dL)	176.9 ± 43.7	186.4 ± 43.4	181.3 ± 41.6	182.3 ± 42.0	183.5 ± 44.5	0.055
Triglycerides (mg/dL)	98.7 ± 75.0	102.0 ± 61.1	103.2 ± 64.4	116.2 ± 110.6	105.8 ± 94.9	0.3
HDL cholesterol (mg/dL)	54.1 ± 14.4	58.3 ± 18.4	55.2 ± 15.8	55.3 ± 15.3	61.5 ± 17.4	0.01
Non-LDL cholesterol (mg/dL)	122.5 ± 42.6	128.2 ± 39.9	126.0 ± 41.0	124.9 ± 41.2	122.8 ± 43.4	0.5
LDL cholesterol	103.8 ± 34.7	107.6 ± 35.5	105.5 ± 36.5	103.1 ± 31.2	103.3 ± 37.6	0.7
BMI (kg/m <sup>2</sup> )	22.8 ± 3.4	23.9 ± 3.8	24.3 ± 3.5	24.4 ± 5.1	25.5 ± 4.6	< 0.001
Overweight or obesity, <i>n</i> (%) <sup>1</sup>	338 (33.5)	153 (35.2)	56 (38.1)	36 (44.4)	25 (49.0)	0.004
Retinopathy, yr (%) <sup>3</sup>						< 0.001
Absent	649 (66.9)	154 (73.3)	104 (77)	60 (76.5)	32 (65.3)	
Non-proliferative	50 (6.7)	36 (17.1)	15 (11.1)	11 (14.1)	10 (20.4)	
Proliferative	48 (6.4)	20 (9.5)	16 (11.9)	7 (9.0)	7 (14.3)	
Nephropathy, yr (%) <sup>4</sup>						0.08
Absent	527 (70.7)	128 (60.7)	91 (67.4)	49 (62.8)	30 (61.2)	
Microalbuminuria	90 (12.1)	43 (20.4)	17 (12.6)	12 (18.8)	8 (28.0)	
Clinical nephropathy	23 (3.1)	9 (4.3)	8 (5.9)	3 (4.7)	2 (5.0)	
Macrovascular complications, yes (%) <sup>5,6</sup>	23 (3.1)	16 (7.6)	13 (9.6)	5 (6.4)	7 (14.3)	< 0.001

<sup>1</sup>The *P* value is related to the comparison among all groups (ANOVA); <sup>2</sup>African-Brazilians, Mulattos, Asians, Native Indians; <sup>3,4,5</sup>Retinopathy, nephropathy and macrovascular complications were considered in patients with criterion to be screened (duration of diabetes ≥ 5 years, *n* = 1219); <sup>6</sup>Overweight or obesity were considered together. The data are presented as *n* (%) mean ± SD or median (minimum/maximum). HbA1c: Glycated hemoglobin; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; BMI: Body mass index.

model with hypertension as the dependent variable the OR for age was 1.041 (95%CI: 1.021-1.061, *P* < 0.001) for duration of diabetes was 1.031 (95%CI: 1.012-1.056, *P* < 0.005), for BMI was 1.069 (95%CI: 1.029-1.110, *P* = 0.005), and for plasma creatinine level was 2.280 (95%CI: 1.722-3.017, *P* < 0.001). The other independent variables did not reach statistical significance.

A small variability attributable to centers was noted only

for macrovascular disease with a variance and standard error of 0.376 (0.276).

## DISCUSSION

Our study showed an association between parity with retinopathy, macrovascular disease and hypertension that disappeared after adjustment for variables that

**Table 3** Effect of parity on body mass index evaluated by random intercept multivariate linear regression and adjusted for clinical and demographic data

Variable	Model 1	Model 2	Model 3	P value
Parity				
Nulliparous (reference)				
1	0.400 (0.278)	0.291 (0.276)	0.291 (0.277)	NS
2	0.366 (0.350)	0.320 (0.347)	0.326 (0.350)	NS
3	0.171 (0.456)	0.119 (0.453)	0.111 (0.454)	NS
≥ 4	1.013 (0.563)	1.029 (0.558)	1.051 (0.560)	0.06
Age	0.081 (0.014)	0.069 (0.014)	0.069 (0.014)	NS
Duration of diabetes (yr)	0.007 (0.015)	0.011 (0.015)	0.013 (0.015)	NS
Insulin dose (U/kg per day)		-1.237 (0.253)	-1.239 (0.254)	(< 0.001)
Metformin use (yr)		-0.960 (0.678)	-0.994 (0.679)	NS
Economic status (classes)				NS
High (reference)				
Medium			0.677 (0.392)	
Low			0.551 (0.386)	
Very low			0.353 (0.397)	
Ethnicity (non-caucasian)			-0.028 (0.201)	NS
Intercept	23.233 (0.166)	23.281 (0.171)	23.814 (0.377)	
Variability attributable to centers				
Variance	0.296 (0.148)	0.340 (0.161)	0.321 (0.155)	
Variability attributable to patients				
Variance	12.475 (0.456)	12.251 (0.448)	12.226 (0.447)	
-2 × loglik	8.192.640	8167.357	8.163.366	

Data are presented as B coefficient or variance (standard error); continuous independent variables are centered on the mean. Model 1: Adjusted for age and duration of diabetes; Model 2: Adjusted for age, duration of diabetes, insulin dose and metformin use; Model 3: Adjusted for age, duration of diabetes, insulin dose, metformin use, economic status and ethnicity. NS: Not significant.

could influence these outcomes, such as age, duration of diabetes, plasma creatinine levels, HbA1c, daily insulin dose and use of metformin. However a borderline effect of parity upon BMI was observed.

The impact of pregnancy and parity on the appearance of diabetes-related chronic complications or the progression of its course is still a matter of controversy<sup>[2]</sup>. Some studies have found no difference in the prevalence of diabetes-related chronic complications between nulliparous and parous women<sup>[24]</sup>, less progression of retinopathy in multiparous than in nulliparous women<sup>[15]</sup> and even a limitations of the progression of nephropathy and retinopathy probably due to a better glycemic control found in parous women compared to nulliparous women<sup>[14]</sup>.

An unexpected finding was that a lower daily insulin dose was associated with a higher BMI. The use of metformin had no effect on BMI probably because the majority of women with increased BMI were under the use of metformin; the use of metformin in patients with T1D has not its clear benefits well established but a decrease in insulin daily dose has generally been described<sup>[25]</sup>.

Nevertheless, it is important to emphasize that the absence of data on body weight before each pregnancy and consequently the weight gain during each pregnancy does not allow us to take any conclusions about this relationship. However, recently a study<sup>[26]</sup> has shown that around 20% of T1D patients have been diagnosed with overweight/obesity. Although weight before the diagnosis of diabetes was not recorded in our sample,

more than one-third of the nulliparous women had overweight or obesity. Overweight and obesity are related to insulin resistance which is strongly associated with cardiovascular disease<sup>[27]</sup>. We have found no effect of parity on cardiovascular disease in our study.

Considering microvascular complications, retinopathy was the most prevalent diabetes-related chronic complication associated with parity but after adjustments for other clinical and demographic variables this association did not show to be significant. Indeed, the most significant variables related to retinopathy were duration of diabetes and the presence of hypertension. The majority of the studies relating pregnancy with retinopathy were prospective and the results were controversial<sup>[1,3,4]</sup>. The DCCT<sup>[1]</sup> and the Eurodiab<sup>[14]</sup> compared women with incident pregnancies during the study period with women who did not conceive. The DCCT study showed a transient worsening of retinopathy, which disappeared 12 mo post-partum and the Eurodiab study did not find any relationship between retinopathy and pregnancy. Indeed, in the Eurodiab study<sup>[14]</sup> the duration of diabetes and the level of HbA1c were the most important predictors of the occurrence of retinopathy. Two other recent studies showed that progression of sight-threatening retinopathy during pregnancy some years post-partum was related to duration of diabetes, to the presence of macular edema and higher blood pressure levels during pregnancy but not to HbA1c levels<sup>[13,28]</sup>. Rosenn *et al.*<sup>[29]</sup> performed a large retrospective study with 776 nulliparous women and 582 parous women with T1D and have found an inverse association with parity and the presence of retinopathy.

Considering nephropathy, our data is in accordance with the findings of Reece *et al.*<sup>[8]</sup> that conducted a study with 31 pregnancies complicated by nephropathy and have found a significant increase in maternal blood pressure, proteinuria and nephrotic syndrome in 71% of pregnancies but no adverse effects of pregnancy on the natural course of the underlying renal disease. Miodovnik *et al.*<sup>[30]</sup> have followed a group of 182 pregnant women with T1D, with and without nephropathy. They have found that pregnancy does not increase the risk of nephropathy and does not accelerate its progression.

These studies regarding the progression of retinopathy and nephropathy were prospective. So, our results must then be interpreted with caution due to its cross-sectional design, that does not allow us to deny a causal relationship between parity and occurrence/worsening of retinopathy and nephropathy in our population. Nevertheless, many women had already retinopathy and nephropathy and also important risk factors for the development or progression of both complications such as the presence of overweight or obesity, hypertension, as described in other studies<sup>[8,10-13,29,30]</sup>.

We should also take in account that for hypertension parity did not reach statistical significance in multivariate analysis. Indeed, age, duration of diabetes BMI, ethnicity (Caucasian) and plasma creatinine levels were the most important factors.

The main strength of our large sample size is that it represents the diverse, young Brazilian population with T1D, with a multi-ethnic and different socioeconomic backgrounds. Also a uniform, standardized recruitment protocol in all participating centers was used.

Finally, some limitations must be addressed in our study. The mean age of our patients is around 25 years, which could represent a short time frame to the appearance of diabetes-related chronic complications. Additionally, we do not have data about how long patients had the diagnosis of diabetes at the time of each pregnancy, occurrence of stillbirth, prematurity, neonatal mortality and no information concerning screening for retinopathy and nephropathy during pregnancies.

In conclusion, our data did not find an effect of parity upon diabetes-related chronic complications and hypertension, but a borderline effect on BMI. These findings should allow us not to discourage women without severe and progressive diabetes-related complications to become pregnant if they reach and maintain a good glycemic control. Further prospective studies must be addressed to clarify the mechanisms underlying the relationship between parity, and the appearance or worsening of diabetes comorbidities and the effect of parity on diabetes-related chronic complications.

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## COMMENTS

### Background

It is generally believed that pregnancy itself or an increasing number of pregnancies might worsen diabetes-related chronic complications.

### Research frontiers

So far, the mechanisms linking pregnancy to both chronic complications are still unclear and controversial.

### Innovations and breakthroughs

For a long time women with diabetes have been discouraged to become pregnant regarding the possibility that an already existing complication might worsen and new complications might appear during pregnancy. This study has shown no relationship between higher parity and the worsening or appearance of diabetes-related chronic complications.

### Applications

This study showed an association between parity with retinopathy, macrovascular disease and hypertension that disappeared after adjustment for variables that could influence these outcomes, such as age, duration of diabetes, plasma creatinine levels, HbA1c, daily insulin dose and use of metformin.

### Peer-review

The manuscript is well written, and the study results and evaluation means are well defined in detail. It provides additional information on a controversial area, while suitably mentioning the shortcomings of the study as well. It will be of good concern to clinicians/researchers working on this subject, while further research will still be needed for clarification of the mentioned links, as the authors also mention.

## REFERENCES

- 1 **The Diabetes Control and Complications Trial Research Group.** Effect of pregnancy on microvascular complications in the diabetes control and complications trial. The Diabetes Control and Complications Trial Research Group. *Diabetes Care* 2000; **23**: 1084-1091 [PMID: 10937502 DOI: 10.2337/diacare.23.8.1084]
- 2 **Väärasmäki M, Anttila M, Pirttiäho H, Hartikainen AL.** Are recurrent pregnancies a risk in Type 1 diabetes? *Acta Obstet Gynecol Scand* 2002; **81**: 1110-1115 [PMID: 12519106 DOI: 10.1034/j.1600-0412.2002.811203.x]
- 3 **Serup L.** Influence of pregnancy on diabetic retinopathy. *Acta Endocrinol Suppl* (Copenh) 1986; **277**: 122-124 [PMID: 3464145 DOI: 10.1530/acta.0.111s0122]
- 4 **Horvat M, Maclean H, Goldberg L, Crock GW.** Diabetic retinopathy in pregnancy: a 12-year prospective survey. *Br J Ophthalmol* 1980; **64**: 398-403 [PMID: 7387964 DOI: 10.1136/bjo.64.6.398]
- 5 **Jovanovic-Peterson L, Peterson CM.** Diabetic retinopathy. *Clin Obstet Gynecol* 1991; **34**: 516-525 [PMID: 1934703 DOI: 10.1097/00003081-199134030-00005]
- 6 **Lapolla A, Cardone C, Negrin P, Midena E, Marini S, Gardellino C, Bruttomesso D, Fedele D.** Pregnancy does not induce or worsen retinal and peripheral nerve dysfunction in insulin-dependent diabetic women. *J Diabetes Complications* 1998; **12**: 74-80 [PMID: 9559484 DOI: 10.1016/S1056-8727(97)00002-0]
- 7 **Ringholm L, Vestgaard M, Laugesen CS, Juul A, Damm P, Mathiesen ER.** Pregnancy-induced increase in circulating IGF-I is associated with progression of diabetic retinopathy in women with type 1 diabetes. *Growth Horm IGF Res* 2011; **21**: 25-30 [PMID: 21212010 DOI: 10.1016/j.ghir.2010.12.001]
- 8 **Reece EA, Coustan DR, Hayslett JP, Holford T, Coulehan J, O'Connor TZ, Hobbins JC.** Diabetic nephropathy: pregnancy performance and fetomaternal outcome. *Am J Obstet Gynecol* 1988; **159**: 56-66 [PMID: 3394754 DOI: 10.1016/0002-9378(88)90494-2]
- 9 **Dahl-Jørgensen K, Brinchmann-Hansen O, Hanssen KF, Sandvik**

- L, Aagaenaes O. Rapid tightening of blood glucose control leads to transient deterioration of retinopathy in insulin dependent diabetes mellitus: the Oslo study. *Br Med J* (Clin Res Ed) 1985; **290**: 811-815 [PMID: 3919804 DOI: 10.1136/bmj.290.6471.811]
- 10 **Klemetti MM**, Teramo K, Nuutila M, Tikkanen M, Hiilesmaa V, Laivuori H. Blood pressure levels but not hypertensive complications have increased in Type 1 diabetes pregnancies during 1989-2010. *Diabet Med* 2013; **30**: 1087-1093 [PMID: 23659525 DOI: 10.1111/dme.12224]
  - 11 **Persson M**, Pasupathy D, Hanson U, Westgren M, Norman M. Pre-pregnancy body mass index and the risk of adverse outcome in type 1 diabetic pregnancies: a population-based cohort study. *BMJ Open* 2012; **2**: e000601 [PMID: 22334581 DOI: 10.1136/bmjopen-2011-000601]
  - 12 **Wender-Ozegowska E**, Zawiejska A, Michalowska-Wender G, Iciek R, Wender M, Brazert J. Metabolic syndrome in type 1 diabetes mellitus. Does it have any impact on the course of pregnancy? *J Physiol Pharmacol* 2011; **62**: 567-573 [PMID: 22204805]
  - 13 **Gordin D**, Kaaja R, Forsblom C, Hiilesmaa V, Teramo K, Groop PH. Pre-eclampsia and pregnancy-induced hypertension are associated with severe diabetic retinopathy in type 1 diabetes later in life. *Acta Diabetol* 2013; **50**: 781-787 [PMID: 22955518 DOI: 10.1007/s00592-012-0415-0]
  - 14 **Chaturvedi N**, Stephenson JM, Fuller JH. The relationship between pregnancy and long-term maternal complications in the EURODIAB IDDM Complications Study. *Diabet Med* 1995; **12**: 494-499 [PMID: 7648822 DOI: 10.1111/j.1464-5491.1995.tb00530.x]
  - 15 **Kaaja R**, Sjöberg L, Hellsted T, Immonen I, Sane T, Teramo K. Long-term effects of pregnancy on diabetic complications. *Diabet Med* 1996; **13**: 165-169 [PMID: 8641123 DOI: 10.1002/(SICI)1096-9136(199602)13:2<165::AID-DIA61>3.0.CO;2-E]
  - 16 **Gomes MB**, Coral M, Cobas RA, Dib SA, Canani LH, Nery M, de Freitas MC, Faria M, Felício JS, da Silva SC, Pedrosa H, Costa e Forti A, Rea RR, Pires AC, Montenegro Junior R, Oliveira JE, Rassi N, Negrato CA. Prevalence of adults with type 1 diabetes who meet the goals of care in daily clinical practice: a nationwide multicenter study in Brazil. *Diabetes Res Clin Pract* 2012; **97**: 63-70 [PMID: 22397904 DOI: 10.1016/j.diabres.2012.02.008]
  - 17 **American Diabetes Association**. Clinical Practice Recommendations. *Diabetes Care* 2013; **34**: S11-S66
  - 18 **World Health Organization**. Obesity: preventing and managing the global epidemic. Report of a World Health Organization Consultation. Geneva: World Health Organization, WHO Obesity Technical Report Series, 2000: 284
  - 19 **Friedewald WT**, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; **18**: 499-502 [PMID: 4337382]
  - 20 **Instituto Brasileiro de Geografia e Estatística (IBGE)**. Censo 2000. [accessed 2008 Aug]. Available from: URL: <http://www.ibge.gov.br/censo/>
  - 21 **Malerbi DA**, Franco LJ. Multicenter study of the prevalence of diabetes mellitus and impaired glucose tolerance in the urban Brazilian population aged 30-69 yr. The Brazilian Cooperative Group on the Study of Diabetes Prevalence. *Diabetes Care* 1992; **15**: 1509-1516 [PMID: 1468278 DOI: 10.2337/diacare.15.11.1509]
  - 22 **ABEP**. Brazilian Economic classification criteria, 2010. [accessed 2008 Aug]. Available from: URL: <http://www.abep.org/novo/Content.aspx?SectionID=84>
  - 23 **Rasbash J**, Charlton C, Browne WJ, Healy M, Cameron B. 2009 MLwiN Version 2.1. Bristol: Centre for Multilevel Modelling, University of Bristol, 2009
  - 24 **Hemachandra A**, Ellis D, Lloyd CE, Orchard TJ. The influence of pregnancy on IDDM complications. *Diabetes Care* 1995; **18**: 950-954 [PMID: 7555555 DOI: 10.2337/diacare.18.7.950]
  - 25 **Vella S**, Buetow L, Royle P, Livingstone S, Colhoun HM, Petrie JR. The use of metformin in type 1 diabetes: a systematic review of efficacy. *Diabetologia* 2010; **53**: 809-820 [PMID: 20057994 DOI: 10.1007/s00125-009-1636-9]
  - 26 **Newfield RS**, Cohen D, Capparelli EV, Shragg P. Rapid weight gain in children soon after diagnosis of type 1 diabetes: is there room for concern? *Pediatr Diabetes* 2009; **10**: 310-315 [PMID: 19067888 DOI: 10.1111/j.1399-5448.2008.00475.x]
  - 27 **Matheus AS**, Tibiriçá E, da Silva PB, de Fátima Bevilacqua da Matta M, Gomes MB. Uric acid levels are associated with microvascular endothelial dysfunction in patients with Type 1 diabetes. *Diabet Med* 2011; **28**: 1188-1193 [PMID: 21658123 DOI: 10.1111/j.1464-5491.2011.03349.x]
  - 28 **Vestgaard M**, Ringholm L, Laugesen CS, Rasmussen KL, Damm P, Mathiesen ER. Pregnancy-induced sight-threatening diabetic retinopathy in women with Type 1 diabetes. *Diabet Med* 2010; **27**: 431-435 [PMID: 20536515 DOI: 10.1111/j.1464-5491.2010.02958.x]
  - 29 **Rosenn BM**, Miodovnik M. Pregnancy and complications of diabetes mellitus: maternal and fetal complications, edn 2. In *Diabetes mellitus: A Fundamental and clinical Text*. Philadelphia, PA: Lippincott, Williams, and Wilkins, 2000: 865-866
  - 30 **Miodovnik M**, Rosenn BM, Khoury JC, Grigsby JL, Siddiqi TA. Does pregnancy increase the risk for development and progression of diabetic nephropathy? *Am J Obstet Gynecol* 1996; **174**: 1180-1189; discussion 1189-1191 [PMID: 8623845 DOI: 10.1016/S0002-9378(96)70660-9]

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## Role of bile acids in the regulation of the metabolic pathways

Hiroki Taoka, Yoko Yokoyama, Kohkichi Morimoto, Naho Kitamura, Tatsuya Tanigaki, Yoko Takashina, Kazuo Tsubota, Mitsuhiro Watanabe

Hiroki Taoka, Tatsuya Tanigaki, Department of Environmental Information, Keio University, Fujisawa, Kanagawa 252-0882, Japan

Yoko Yokoyama, Naho Kitamura, Graduate School of Media and Governance, Keio University, Fujisawa, Kanagawa 252-0882, Japan

Kohkichi Morimoto, Division of Endocrinology, Metabolism and Nephrology, Department of Internal Medicine, Keio University School of Medicine, Shinjuku, Tokyo 160-8582, Japan

Yoko Takashina, Research Institute of SFC, Keio University, Fujisawa, Kanagawa 252-0882, Japan

Kazuo Tsubota, Department of Ophthalmology, Keio University School of Medicine, Shinjuku, Tokyo 160-8582, Japan

Mitsuhiro Watanabe, Graduate School of Media and Governance, Department of Environment and Information Studies, Keio University, Fujisawa, Kanagawa 252-0882, Japan

Mitsuhiro Watanabe, Department of Internal Medicine, Keio University School of Medicine, Shinjuku, Tokyo 160-8582, Japan

**Author contributions:** Taoka H, Watanabe M devised the study concept and design; Taoka H, Yokoyama Y, Morimoto K, Watanabe M searched the literature; Taoka H, Yokoyama Y, Morimoto K, Kitamura N, Watanabe M drafted the article; all authors revised the article for important intellectual content; Watanabe M gave final approval for the article.

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**Correspondence to:** Mitsuhiro Watanabe, PhD, Professor of Graduate School of Media and Governance, Professor of Faculty of Environment and Information Studies, Graduate School of Media and Governance, Department of Environment and Information Studies, Keio University, tau42, 5322 Endo, Fujisawa, Kanagawa 252-0882, Japan. [wmitsu@sfc.keio.ac.jp](mailto:wmitsu@sfc.keio.ac.jp)  
**Telephone:** +81-466-493516  
**Fax:** +81-466-493516

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### Abstract

Recent studies have revealed that bile acids (BAs) are not only facilitators of dietary lipid absorption but also important signaling molecules exerting multiple physiological functions. Some major signaling pathways involving the nuclear BAs receptor farnesoid X receptor and the G protein-coupled BAs receptor TGR5/M-BAR have been identified to be the targets of BAs. BAs regulate their own homeostasis *via* signaling pathways. BAs also affect diverse metabolic pathways including glucose metabolism, lipid metabolism and energy expenditure. This paper suggests the mechanism of controlling metabolism *via* BA signaling and demonstrates that BA signaling is an attractive therapeutic target of the metabolic syndrome.

**Key words:** Bile acids; TGR5/M-BAR; Farnesoid X receptor; Glucose metabolism; Energy metabolism; Lipid metabolism; Bariatric surgery; Microbiota; Incretin; Bile acid binding resin

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**Core tip:** Bile acids (BAs) are important molecules that participate in various metabolic pathways. BA signaling mechanisms are attractive therapeutic targets of the metabolic syndrome. In this review, we show the mechanisms of controlling glucose, lipid and energy metabolism *via* BA signaling. Furthermore, the authors also describe how those basic scientific studies have been applied to the clinical setting. Particularly, bile acid binding resin (BABR) originally used to treat hypercholesterolemia also stimulates incretin secretion and improves glucose metabolism. In addition to BABR, the clinical application of farnesoid X receptor and TGR5/M-BAR agonists are ongoing for the treatment of metabolic syndrome. The effects of bariatric surgery on glycemic control are also associated with BA metabolism.

Taoka H, Yokoyama Y, Morimoto K, Kitamura N, Tanigaki T, Takashina Y, Tsubota K, Watanabe M. Role of bile acids in the regulation of the metabolic pathways. *World J Diabetes* 2016; 7(13): 260-270 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v7/i13/260.htm> DOI: <http://dx.doi.org/10.4239/wjcd.v7.i13.260>

## INTRODUCTION

Bile acids (BAs) are the main constituents of bile and amphipathic molecules, containing both hydrophilic and hydrophobic regions. BAs are synthesized from cholesterol in the liver, stored in the gall bladder, and flow into the small intestine after meal ingestion. Intestinal BAs facilitate digestion and absorption of lipids and fat-soluble vitamins<sup>[1]</sup>.

Recent reports suggest that BAs are responsible not only for the absorption of lipids but also for signal transduction. Some major signaling mechanisms have been identified, including the MAPK pathways, nuclear hormone receptor farnesoid X receptor (FXR)-mediated pathway and G protein-coupled receptor TGR5/M-BAR (also named GPR131)-mediated pathway<sup>[2-5]</sup>. BAs have been demonstrated to be natural ligands of FXR. The main role of the FXR signaling pathway is regulating both enterohepatic circulation and BA biosynthesis to maintain the homeostasis of BA<sup>[6]</sup>. In addition, FXR signaling has been known to regulate lipogenesis gene expression and improve hepatic steatosis<sup>[7]</sup>. Moreover, recent studies have shown that BAs and FXR signaling are associated with the beneficial glycemic effects of bariatric surgery and regulation of autophagy<sup>[8-10]</sup>. BAs also activate TGR5/M-BAR. The TGR5/M-BAR signaling pathway stimulates energy expenditure in both brown adipose tissue (BAT) as well as skeletal muscle<sup>[11]</sup>. Furthermore, TGR5/M-BAR plays a role in hepatic microcirculation as well as cytokine release from macrophages<sup>[12]</sup>. Taken together, BAs not only participate in the digestion and absorption of lipids but also in various metabolic pathways. BA signaling

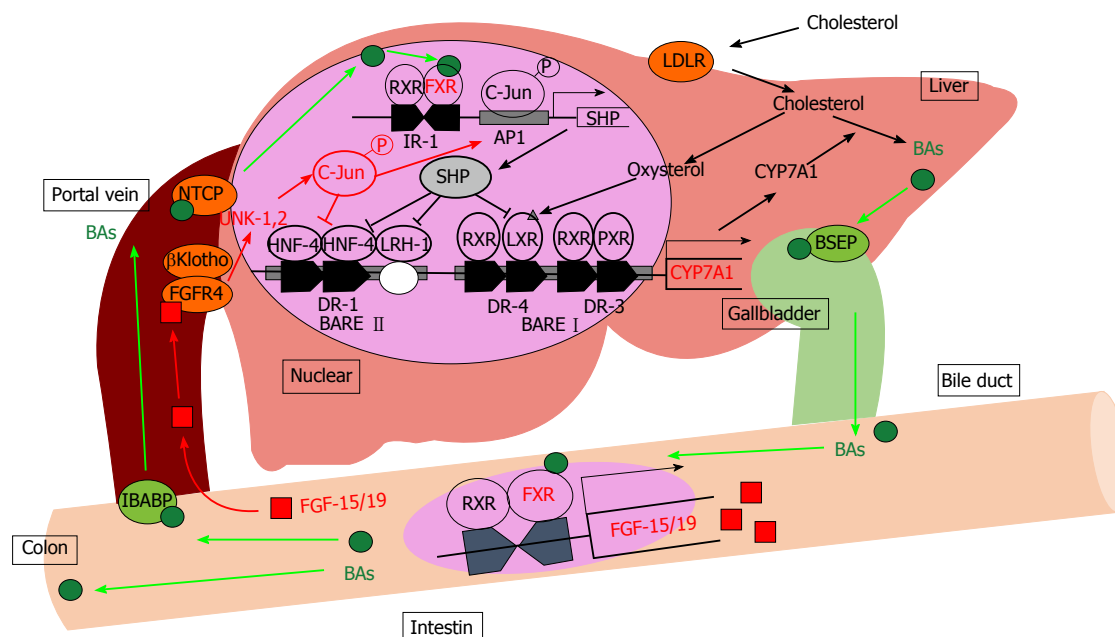
participates in various diseases such as cancer, immune disorders, and metabolic syndrome<sup>[13-15]</sup>. In this review, we summarize the current knowledge of the metabolic regulation mechanisms of BAs and propose BA signaling pathways as a therapeutic target of the metabolic syndrome.

## BILE ACIDS METABOLISM

The majority of synthesized BAs are secreted into the bile and kept in the gallbladder. When food enters the gastrointestinal tract, bile flows into the small intestine, and are efficiently absorbed by active transport and passive diffusion in the terminal ileum. BAs are then transported again to the liver through the portal vein and re-uptaken at the sinusoidal membranes of hepatocytes. These BAs are then secreted into the bile again; each BA molecule can complete 4-12 cycles of circulation per day<sup>[16]</sup>.

BA synthesis has two differential pathways: The "classic (or neutral) pathway" and the "alternative (or acidic) pathway". In the classic pathway, the enzyme cholesterol-7 $\alpha$ -hydroxylase (CYP7A1) hydroxylates the C7 $\alpha$  position during the first step. In the alternate pathway, the enzyme sterol-27 $\alpha$ -hydroxylase (CYP27A1) first hydroxylates the C27 position. The classic pathway seems more important than the alternative pathway because the classic pathway is responsible for maintaining cholesterol homeostasis by controlling BA synthesis<sup>[17]</sup>. The rate-limiting enzyme CYP7A1 converts cholesterol to 7 $\alpha$ -hydroxycholesterol, and other enzymes including sterol-12 $\alpha$ -hydroxylase (CYP8B1), 25-hydroxycholesterol-7 $\alpha$ -hydroxylase (CYP7B1) and CYP27A1 convert 7 $\alpha$ -hydroxycholesterol to primary BAs, including cholic acid (CA) and chenodeoxycholic acid (CDCA)<sup>[18]</sup>. CYP8B1 controls the production of CA, and CA regulates the CA/CDCA ratio in humans or the CA/MCA ratio in mice by mediating feedback regulation<sup>[19]</sup>. Regulation of this ratio is important because previous studies demonstrated that the ratio of CA/CDCA is associated with liver diseases in humans<sup>[20]</sup>. For example, this ratio is decreased in patients with liver cirrhosis and hepatic cancer but is increased in cholestasis. Most of the BAs are conjugated to glycine or taurine, and the ratio of BAs conjugated to taurine and glycine differ depending on the animal species. In humans, the ratio of BAs conjugated to taurine and glycine are approximately 1:2, and most BAs are conjugated with taurine in mice. BAs inhibit the expression of CYP7A1 and CYP8B1 in liver through several pathways, which are mainly FXR-dependent. BAs activate FXR, leading to the upregulation of a small heterodimer partner (SHP; NR0B2), which suppresses the activity of hepatocyte nuclear factor-4 $\alpha$  (HNF-4 $\alpha$ ; NR2A1), liver X receptor (LXR; Nr1h3) and liver receptor homolog-1 (LRH-1; NR5A2), which are both required for transcriptional induction of BA synthesis enzymes *via* binding to BA-response elements in promoters<sup>[21-23]</sup>. Additionally, the intestinal activation of FXR by BAs causes an increased expression of fibroblast growth





**Figure 1 Bile acid metabolism in the liver.** BAs induce the FXR-SHP-mediated pathway and repress BA synthesis enzyme gene expression such as CYP7A1 and CYP8B1. Synthesized BAs increase the expression of FGF-15/19 in the small intestine. FGF-15/19 signaling induces JNK pathway activation resulting in the repression of CYP7A1 transcription. AP1: Activator protein 1; BAs: Bile acids; BARE: Bile acid response element; BSEP: Bile salt export pump; CYP7A1: Cholesterol-7 $\alpha$ -hydroxylase; DR: Direct repeat element; FGF-15/19: Fibroblast growth factor-15/19; FGFR4: Fibroblast growth factor receptor 4; FXR: Farnesoid X receptor; HNF-4: Hepatocyte nuclear factor; IBABP: Intestinal bile acid-binding protein; IR-1: Inverted repeat element-1; JNK: Jun-N-terminal kinase; LDLR: Low-density lipoprotein receptor; LRH-1: Liver receptor homolog-1; LXN: Liver X receptor; NTCP: Sodium-taurocholate cotransporting polypeptide; PXR: Pregnenolone X receptor; RXR: Retinoid X receptor; SHP: Small heterodimer partner.

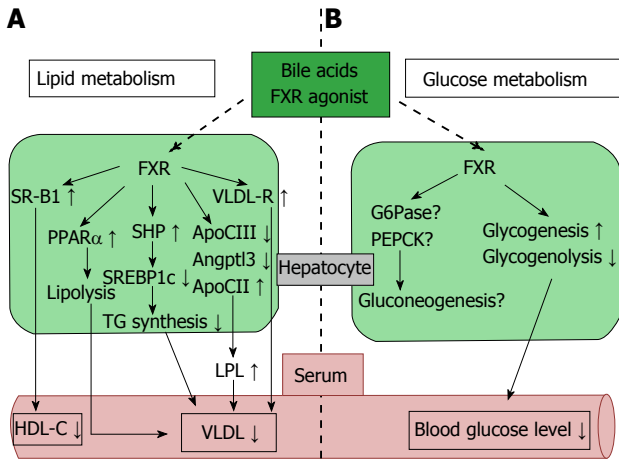
factor (FGF)-15 in rodents and FGF-19 in humans. BAs absorbed in the terminal ileum activate intestinal FXR and induce enterocytic production of FGF-15/19. This FGF-15/19 is passed from the portal vein to the hepatocytes and couples with a receptor, FGF receptor 4 (FGFR4). These signaling pathways *via* FGF-15/19 and FGFR4 induce receptor dimerization, autophosphorylation, and c-Jun N-terminal kinase pathway activation resulting in the repression of CYP7A1 transcription (Figure 1)<sup>[24,25]</sup>. A second BA receptor, TGR5/M-BAR, also contributes to regulation of BA homeostasis. TGR5/M-BAR knockout mice present with a decrease in the BA pool size and the impaired suppression of CYP7A1 expression upon BA administration<sup>[26,27]</sup>. Vitamin D also regulates BAs synthesis. Vitamin D receptor activation induces the expression of FGF-15/19, and BA synthesis is decreased by reducing CYP7A1 expression<sup>[28,29]</sup>. BAs regulate BA homeostasis *via* FXR, TGR5/M-BAR and other signaling pathways primarily by maintaining gene expression of the rate-limiting enzymes CYP7A1 and CYP8B1.

## BILE ACIDS IN GLUCOSE METABOLISM

Previous studies have clarified that BAs affect glucose metabolism. Glucose induces the expression of FXR and CYP7A1, and insulin reduces their expression *in vitro*<sup>[30]</sup>. Further studies have shown that BAs seem to regulate gluconeogenesis, but the mechanisms remain poorly understood. Some studies have indicated that the expression of phosphoenolpyruvate carboxykinase (PEPCK), which is the rate-limiting enzyme of gluconeogenesis,

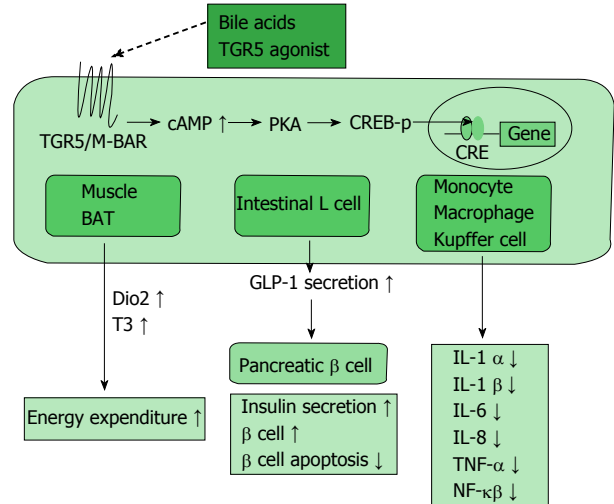
is suppressed by BAs in human liver cancer cells (HepG2 cells) and the mouse liver<sup>[31-33]</sup>. Additionally, enzymes such as glucose 6-phosphatase and fructose 1,6-bisphosphatase 1 which also participate in gluconeogenesis are repressed by BAs<sup>[31]</sup>. These effects are decreased in FXR and SHP knockout mice, which supports the idea that BAs suppress gluconeogenesis in a FXR-SHP-dependent manner<sup>[33]</sup>; however, others have reported that FXR-dependent signaling induces PEPCK expression and increases gluconeogenesis in primary hepatocytes and rat hepatoma cell lines<sup>[34]</sup>. In terms of glycogen synthesis, BAs increase hepatic glycogen synthesis and storage, resulting in decreased blood glucose levels in an FXR-dependent manner (Figure 2B)<sup>[35]</sup>. A previous study demonstrated that long-term FXR activation (3 mo) with a synthetic FXR agonist, GW4064, worsened glucose intolerance and insulin resistance in high-fat fed C57BL/6J mice<sup>[33,36]</sup>. The mechanism behind the effects of GW4064 is lowering the BA pool size following FXR activation. Some reports have suggested that short-term (10 d) FXR activation by the synthetic FXR agonist GW4064 reduced glycolytic gene expression and improved insulin resistance in *ob/ob* or *db/db* mice<sup>[35,37]</sup>. In contrast, the difference of the GW4064 administration period may lead to the opposite result. Long-term administration of BAs, the endogenous natural ligands of FXR, did not decrease the BA pool size and subsequently improved glucose intolerance and insulin resistance<sup>[36]</sup>.

BA administration improved metabolism including glucose tolerance and insulin resistance. The beneficial



**Figure 2 Farnesoid X receptor-dependent metabolic regulation in the liver.** Hepatic FXR signaling regulates lipid and glucose metabolism. A: FXR signaling reduces lipogenesis (SREBP1c) and induces fatty acid  $\beta$  oxidation (PPAR $\alpha$ ) and plasma TG clearance (LPL and VLDL-R), resulting in decreased plasma VLDL levels. Plasma HDL-C uptake is also increased by FXR and SRB1 activity; B: FXR signaling up-regulates glycogenesis, down-regulates glycogenolysis, and reduces blood glucose levels. Hepatic FXR signaling is also associated with gluconeogenesis, but the controlling mechanism is still unclear. Angptl3: Angiopoietin-like protein 3; ApoC II/C III: Apolipoprotein-C II/C III; FXR: Farnesoid X receptor; G6Pase: Glucose-6-phosphatase; HDL-C: High density lipoprotein-cholesterol; LPL: Lipoprotein lipase; PEPC: Phosphoenolpyruvate carboxykinase; PPAR $\alpha$ : Peroxisome proliferator-activated receptor  $\alpha$ ; SR-B1: Scavenger receptor-B1; SREBP1c: Sterol regulatory element-binding protein 1c; TG: Triglyceride; VLDL-R: Very low density lipoprotein-receptor.

effects of BAs, such as a decrease in gluconeogenesis and increase in glycogen synthesis, seem to occur not only through FXR signaling but also through a number of other signaling molecules, such as TGR5/M-BAR. BAs stimulate incretins, such as glucagon-like peptide-1 (GLP-1; Figure 3). GLP-1 is secreted by dietary stimulation from enteric L cells and promotes insulin secretion by binding to the GLP-1 receptor in the pancreatic  $\beta$  cell. Further, GLP-1 maintains pancreatic function, and GLP-1 receptor agonists have been developed for the treatment of diabetes<sup>[38]</sup>. TGR5/M-BAR signaling causes GLP-1 secretion in mouse enteroendocrine STC-1 cells<sup>[39]</sup>. Moreover, 6-ethyl-23(S)-methylcholic acid (6EMCA or INT-777<sup>[40]</sup>), a semisynthetic TGR5/M-BAR agonist, stimulates the secretion of GLP-1 in both mouse and human enteroendocrine cells. In the present study, knock-down of TGR5/M-BAR by shRNA decreased 6EMCA-induced secretion of GLP-1 in STC-1 cells<sup>[41]</sup>. The natural TGR5/M-BAR agonist oleanolic acid also improves the metabolism of glucose<sup>[42]</sup>. This evidence indicates the importance of TGR5/M-BAR in GLP-1 secretion. An *in vivo* study with TGR5/M-BAR transgenic and TGR5/M-BAR knockout mice strongly supports the relationship between TGR5/M-BAR and GLP-1 secretion<sup>[43]</sup>. Considering the current mechanism, TGR5/M-BAR activation increases cAMP levels and the ATP/ADP ratio, which then leads to depolarization of the plasma membrane as well as  $\text{Ca}^{2+}$  mobilization, resulting in increased GLP-1 release<sup>[41]</sup>. Additionally, a human genetic study revealed an association between a single nucleotide polymorphism,



**Figure 3 TGR5/M-BAR-dependent metabolic regulation.** TGR5/M-BAR activation leads to increased intracellular cAMP levels, the activation of PKA and induction of CREB phosphorylation. This series of signaling activity induces the expression of genes bearing CRE and exists in various tissues. TGR5/M-BAR signaling induces energy expenditure in the muscle and BAT, increases GLP-1 secretion in the intestinal L cell, and reduces inflammatory cytokine release in immune cells. CREB-p: cAMP response element-binding protein phosphorylation; Dio2: Deiodinase iodothyronine type II; T3: Tri-iodothyronine; BAT: Brown adipose tissue; GLP-1: Glucagon-like peptide-1.

rs3731859, of the *TGR5/M-BAR* gene and various metabolic indexes including BMI, waist circumference, intramyocellular lipid, and fasting serum GLP-1 levels<sup>[44]</sup>. Hence, these findings suggested that GLP-1 secretion was stimulated by TGR5/M-BAR signaling *in vivo*. BAs and TGR5/M-BAR could become therapeutic targets of diabetes.

## BILE ACIDS IN LIPID METABOLISM

BAs are important in regulating triglyceride (TG) metabolism as well as cholesterol metabolism. The relationship between BAs and TG was first reported in the treatment of gallstones with CDCA. CDCA treatment decreased the serum TG level in patients with gallstones<sup>[45]</sup>. In fact, BAs or a synthetic FXR agonist affected TG metabolism *via* several mechanisms including the FXR-mediated pathway. The target of FXR, SHP, suppressed up-regulation of sterol regulatory element-binding protein-1c (SREBP-1c), the master regulator of fatty acid and TG synthesis, to reduce the expression of the lipogenic genes such as acetyl CoA synthetase, acetyl CoA carboxylase, stearoyl CoA desaturase 1, and fatty acid synthase<sup>[7,46]</sup>. In addition, the TG-lowering effects were attenuated in SHP knockout mice, indicating that lipogenesis mediated by SREBP-1c is suppressed in an FXR-SHP-dependent manner<sup>[7]</sup>. Additionally, FXR activation by BAs increases expression of apolipoprotein (Apo) C II. Apo C II activates lipoprotein lipase, which in turn stimulates TG hydrolysis in very low density lipoprotein (VLDL) and chylomicrons, and also facilitates the clearance of TG from the serum<sup>[47]</sup>. The expression of ApoCIII and angiopoietin-like protein 3, which inhibits

the activity of lipoprotein lipase, were repressed by FXR stimulation with BAs<sup>[48-50]</sup>. In addition, FXR induces the expression of the VLDL receptor, which acts to clear plasma TG (Figure 2A)<sup>[51]</sup>.

BAs also represses the expression of microsomal triglyceride transfer protein (MTP) and ApoB in an FXR-independent manner to suppress the formation of VLDL and chylomicrons<sup>[52]</sup>. Not only VLDL but also high density lipoprotein (HDL) clearance are suggested to be subject to modulation by BAs. Expression of scavenger receptor B1 (SRB1), a molecule in charge of hepatic uptake of HDL, is decreased, and HDL-C (HDL-cholesterol) is elevated in FXR knockout mice<sup>[53]</sup>. In addition, the administration of an FXR ligand increases hepatic SRB1 expression and decreases HDL-C levels (Figure 2A)<sup>[54]</sup>.

BAs control other major regulators of lipid metabolism such as proliferator-activated receptor  $\alpha$  (PPAR $\alpha$ ) and pyruvate dehydrogenase kinase-4 (PDK4). The nuclear receptor PPAR $\alpha$ , which is activated by free fatty acids (FFA), decreases serum TG levels and exerts an important role for controlling enzymes participating in fatty acid  $\beta$  oxidation (Figure 2A)<sup>[55]</sup>. A study suggested that BAs directly regulate PPAR $\alpha$  through FXR in humans, but not in mice<sup>[56]</sup>. PDK4 is also up regulated by BAs in an FXR-dependent manner, resulting in inactivation of pyruvate dehydrogenase, decreased glycolysis and increased oxidation of fatty acid  $\beta$ <sup>[57]</sup>. BAs are also associated with atherosclerosis<sup>[58,59]</sup>. Treatment with TGR5/M-BAR agonist INT-777 represses the activation of inflammatory cytokines such as NF- $\kappa$ B and inhibits foam cell formation and subsequent atherosclerotic plaques. In addition, INT-777 does not inhibit atherosclerosis in TGR5/M-BAR knockout mice, supporting the anti-atherosclerotic effect of TGR5/M-BAR (Figure 3)<sup>[58]</sup>.

## BILE ACIDS IN ENERGY METABOLISM

BAs have been reported to stimulate adaptive thermogenesis and energy expenditure *via* TGR5/M-BAR (Figure 3)<sup>[11]</sup>. TGR5/M-BAR activation leads to increased intracellular cAMP levels, activation of PKA and induction of CREB phosphorylation. This series of signaling activity induces the expression of genes bearing a cAMP responsive element and exists in various tissues<sup>[60,61]</sup>.

In the BAT, TGR5/M-BAR stimulation increases the intracellular cAMP level and induces cAMP-dependent iodothyronine deiodinase type 2 (*Dio2*) expression, which converts inactive thyroxine (T4) to active 3,5,3'-triiodothyronine (T3) to evoke increased energy expenditure<sup>[11]</sup>. *Dio2* increases the nuclear T3 level without various unwanted side effects caused by increased blood T3 levels. Only 20% of nuclear T3 is produced and secreted from the human thyroid gland, and the remaining nuclear T3 is supplemented from other tissues. *Dio2* supplies approximately 50% of the T3 in the nucleus including the BAT<sup>[62]</sup>. The BAT is one of the most important targets of BAs to increase energy expenditure. Although BAT had been regarded as a tissue only in newborn infants, recent studies with FDG-

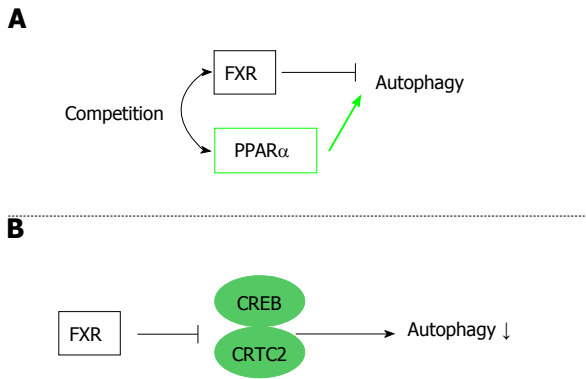
PET revealed the existence of BAT in the shoulders and neck in adult humans, especially with brief cold exposure<sup>[63-65]</sup>. Furthermore, several groups have shown the importance of BAT in adult humans. In healthy patients, the amount of BAT is large and its activity is high but are reduced in obese patients<sup>[66-68]</sup>. In addition, TGR5/M-BAR and *Dio2* are co-expressed in skeletal muscle in humans, which suggests the presence of a thermogenic mechanism in humans<sup>[11]</sup>. Moreover, a recent study found another type of adipocyte termed "beige" cells which are derived from white adipose tissue. These adipocytes also respond to cyclic AMP stimulation with high uncoupling protein (UCP) 1 expression and respiration rates similar to BAT cells<sup>[69,70]</sup>. These accumulating findings suggest a therapeutic approach to improve obesity and metabolic syndrome by increasing energy expenditure through TGR5/M-BAR stimulation.

## BILE ACIDS IN AUTOPHAGY

Autophagy is an evolutionarily conserved catabolic system that maintains energy homeostasis by recycling nutrients in the fasted state. Recent studies have revealed that FXR stimulation suppresses autophagy in the liver. FXR and peroxisome PPAR $\alpha$  competitively bind to the promoter regions of autophagic genes, and these receptors show conflicting effects on transcription<sup>[8]</sup>. In the liver, PPAR $\alpha$  activation under fasted conditions promotes autophagic lipolysis, while FXR activation under fed conditions suppresses autophagy. That is, PPAR $\alpha$  and FXR competitively regulate autophagy based on the nutritional condition (Figure 4A). Another study also revealed that FXR and cAMP response element-binding protein (CREB), which is a transcriptional activator under starvation, competitively regulate autophagy in the liver<sup>[9]</sup>. In the fasted condition, CREB binding to its coactivator CREB regulated the transcription coactivator 2 (CRTC2) to induce CRTC2 activity and subsequent autophagic-related gene expression. Additionally, FXR stimulation caused by feeding disrupts the functional CREB-CRTC2 complex and downregulates autophagy (Figure 4B). In any case, there is no doubt that FXR acts as a suppresser of autophagy.

## ROLES OF BILE ACIDS IN THE GASTROINTESTINAL TRACT

Intestinal FXR has been recently identified as a possible target for improving metabolic syndrome. Intestinal FXR activation induces the expression of FGF-15/19, and several studies have demonstrated that FGF-15/19 affects glucose and energy homeostasis. FGF-19 transgenic mice showed increased hepatic  $\beta$  oxidation, reduced adipose tissue weight, and improved glucose tolerance and insulin sensitivity<sup>[71]</sup>. In mice, hepatic acetyl-CoA carboxylase 2 (ACC2) mRNA was decreased, and the mass of the BAT was increased. ACC2 exists at the mitochondrial membrane and converts acetyl-CoA



**Figure 4 Autophagy regulation by the farnesoid X receptor.** FXR is associated with regulation of autophagy. Two different mechanisms are reported. A: FXR and PPAR $\alpha$  competitively bind to the promoter regions of autophagic genes, and FXR activation suppresses autophagy; B: FXR stimulation disrupts the functional CREB–CRTC2 complex and suppresses autophagy. FXR: Farnesoid X receptor; PPAR $\alpha$ : Peroxisome proliferator-activated receptor  $\alpha$ ; CREB: cAMP response element-binding protein; CRTC2: CREB regulated transcription coactivator 2.

to malonyl-CoA. ACC2 activation results in an elevation of malonyl-CoA levels, which inhibit carnitine palmitoyl transferase-1 (CPT-1) activation<sup>[72]</sup>. CPT-1 transfers FFA from the cytoplasm to the mitochondria and induces fatty acid  $\beta$  oxidation. Thus, the overexpression of FGF-19 suppresses ACC2 mRNA levels, decreases malonyl-CoA levels, activates CPT-1, and thereby increases  $\beta$ -oxidation in the liver. In addition, hyperglycemia is improved upon administration of FGF-19 protein in obese mice<sup>[73]</sup>. Furthermore, activation of intestinal FXR by administration of fexaramine, an FXR agonist, improved obesity and insulin resistance by inducing FGF-15, changing the serum BA composition and stimulating systemic TGR5/M-BAR<sup>[74]</sup>. These results suggest the possibility that metabolic disease is improved through the intestinal FXR-FGF-15/19 signaling pathway (Figure 5B).

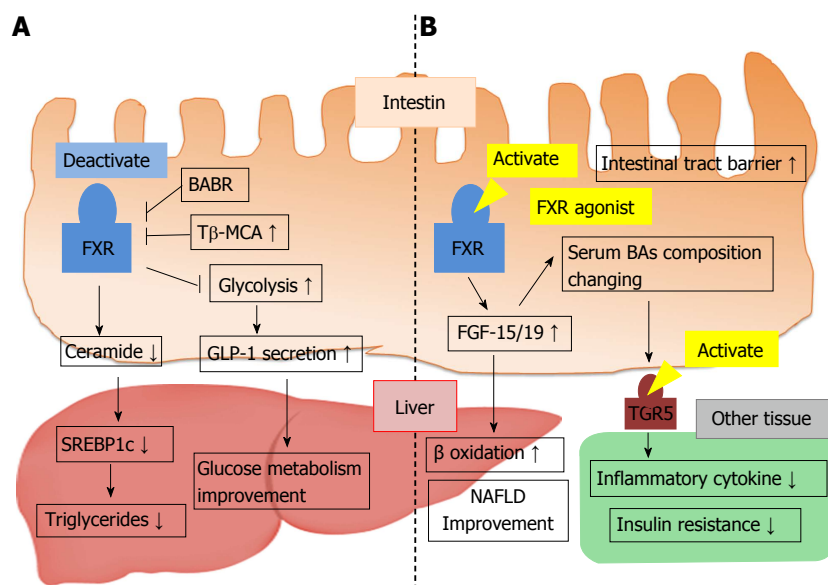
The primary BAs excreted into the intestine become deconjugated BAs and are converted into various secondary BAs by microbial enzymes<sup>[75]</sup>. In germ-free (GF) mice, a decrease in the gut microbiota that facilitate BA deconjugation leads to increased tauro-beta-muricholic acid (T- $\beta$ -MCA). In comparison to conventionally raised mice, FXR-dependent BA synthesis is reduced in GF mice. Therefore, T- $\beta$ -MCA is an FXR antagonist, and the microbiota affect bile acid homeostasis *via* the inhibition of intestinal FXR signaling by change in the BA composition<sup>[76]</sup>. In contrast to previous reports, recent studies have noted that alteration of the BA composition by microbiota and inhibition of intestinal FXR activity improved lipid and glucose metabolism. Increased T- $\beta$ -MCA reduced intestinal FXR activation and decreased serum ceramide levels through repression of ceramide synthesis. Decreased ceramide downregulated expression of hepatic SREBP-1c and resulted in an improvement of obesity and nonalcoholic fatty liver disease (NAFLD)<sup>[77-79]</sup>. Additionally, intestinal FXR deactivation may also improve glucose metabolism as well as lipid metabolism. FXR

activation in L cells decreased glycolysis, proglucagon expression and cAMP levels. Thus, GLP-1 production and secretion were inhibited (Figure 5A)<sup>[80]</sup>. Conflicting opinions suggest that microbiota regulation of BA homeostasis and intestinal FXR activation are involved in controlling hepatic lipid accumulation and glucose metabolism. Further studies are needed to clarify the roles of intestinal FXR signaling for improving metabolic diseases.

Bariatric surgery provides another clue to identifying the link between BAs and glucose homeostasis. Bariatric surgery, particularly gastric bypass surgery, is an established modality for obesity and type 2 diabetes mellitus, albeit that the mechanism of its effectiveness remains unclear. Interestingly, an improvement in glycemic control is seen soon after the surgery, when the body weight remains unchanged. Therefore, some of the anti-metabolic syndrome effects of this surgical intervention appear to be independent of body weight reduction. One recent study suggested that BAs might participate in this immediate effect of bariatric surgery. Following gastric bypass, the bile flow is changed, which leads to an increase in plasma BA level and incretin secretion<sup>[81]</sup>. Hormonal factors and the gut microbiota might also be involved in the effects of this surgery. The gut microbiota is responsible for the enteral BA metabolism, and the normal spectrum of gut microbiota is impacted by gastrointestinal surgery. As one example, the predominant presence of *Firmicutes* was reportedly diminished, and other species, such as methanogens and *Prevotellaceae*, were also inhibited after bariatric surgery<sup>[82]</sup>. In addition to these studies, recent research has revealed that FXR is associated with the effect of bariatric surgery<sup>[83]</sup>. Interestingly, in FXR knockout mice, metabolic improvements such as weight loss and improved glucose tolerance were reduced after bariatric surgery. Furthermore, the surgery changed the gut microbial communities differently between wild type and FXR knockout mice. This study suggested that BAs may affect glucose homeostasis *via* FXR signaling and alterations of the gut microbiota after bariatric surgery. Further investigations are expected.

Bile acid binding resin (BABR) is an effective drug for the treatment of hypercholesterolemia by lowering LDL-cholesterol. BABR absorbs BAs in the intestine, thereby preventing their uptake in the ileum, interrupting their enterohepatic circulation, and facilitating their excretion in the feces. The inhibition of the enterohepatic circulation leads to a reduction of the BA pool size, repression of FXR-SHP and FGF-15/19 signaling, and induction of CYP7A1 expression and synthesis of BAs from the cholesterol to maintain the BA pool size. A decrease in intrahepatic cholesterol levels activates SREBP-2, which induces the expression of the LDL receptor (LDLR) to enhance cholesterol uptake, reducing serum cholesterol levels. In addition to lowering the serum cholesterol effect, there is interaction between BABR and glucose





**Figure 5** Conflicting mechanisms of metabolic regulation via intestinal farnesoid X receptor activity. A: FXR activation decreases hepatic TG levels and improves glucose metabolism; B: Intestinal FXR activation of FXR agonist leads to FGF-15/19 production and improves nonalcoholic fatty liver disease. Synthesized FGF-15/19 changes BA metabolism and serum BA composition, which causes TGR5/M-BAR activation, reduced inflammatory cytokine release, and improved insulin resistance. BABR: Bile acid binding resin; FGF-15/19: Fibroblast growth factor-15/19; FXR: Farnesoid X receptor; NAFLD: Nonalcoholic fatty liver disease; SREBP1c: Sterol regulatory element-binding protein 1c; T $\beta$ -MCA: Tauro- $\beta$ -muricholic acid; GLP-1: Glucagon-like peptide-1; TG: Triglyceride.

metabolism<sup>[84]</sup>. In a diet-induced obesity rat model, BABR decreased serum glucose and improved glucose tolerance<sup>[85,86]</sup>. In a clinical trial, cholestyramine, a first generation BABR, improved glycemia by 13% in patients with type 2 diabetes<sup>[87]</sup>. In addition, a second generation BABR also improved glucose clearance and increased serum GIP and GLP-1 levels in patients with type 2 diabetes mellitus<sup>[88]</sup>. These studies clarified that BABR is not absorbed in the body and there are few unwanted side effects. Furthermore, BABR can decrease blood glucose levels only in high glucose situations. As a result, in January 2008, this drug was approved as a therapeutic drug for diabetes by the Food and Drug Administration (FDA) in the United States<sup>[87,89-92]</sup>.

Although how BABR improves diabetes remains unknown, several possible mechanisms have been proposed. BABR-mediated improvement of hepatic insulin sensitivity depends on downregulating the hepatic cholesterol-LXR-IRS2 pathway<sup>[93]</sup>. In addition, BABR induces GLP-1 secretion via the activation of TGR5/M-BAR or GPR40, each being activated by BAs binding with BABR or unabsorbed long-chain fatty acids<sup>[39,94,95]</sup>. Further, BABR affects the make-up of the BA pool and peripheral BAs, which results in the induction of peripheral energy expenditure and improved glucose tolerance<sup>[84]</sup>. The BABR effects of improving diabetes may be explained by the inhibition of intestinal FXR as well as TGR5/M-BAR signaling<sup>[80]</sup>. BABR colesvelam inhibits intestinal FXR activation and improves glucose metabolism by increasing proglucagon gene expression and inducing GLP-1 secretion in *ob/ob* mice<sup>[80]</sup>. These findings suggest that inhibiting FXR in the L cell via BABR could be a new target for diabetes.

## CLINICAL APPLICATION IN BA SIGNALING

Currently, BABR has been approved by the FDA and has been clinically used as a diabetes treatment drug. The association between bariatric surgery and BA homeostasis was confirmed. In addition to BABR and bariatric surgery, other clinical applications based on the mechanism of metabolic control via BA signaling are ongoing. For instance, INT-747 (also named 6-ethyl-CDCA), which is a synthetic FXR agonist, exerts a hepatoprotective effect in patients of primary biliary cirrhosis (PBC)<sup>[96-98]</sup>, and a phase III clinical study has already been completed and confirmed the effect of PBC. In addition to medicine, INT-747 has also entered into a study for NAFLD treatment. A phase II clinical trial for NAFLD has been completed, and an improvement was observed in type 2 diabetes mellitus patients with NAFLD. Clinical trials with TGR5 agonists, such as INT-777, are ongoing, and future studies are expected<sup>[40,41,99]</sup>.

Altogether, these clinical applications will elucidate the BA signaling mechanisms that will lead to the improvement of metabolic disorders including obesity and diabetes.

## CONCLUSION

Today, BAs have become important molecules to control metabolic homeostasis. In this review we discussed the relationship between BA metabolism and signal transmission, such as the FXR and TGR5/M-BAR pathways and the possibility that BAs may improve metabolic diseases. Current evidence shows that BAs regulate lipid,

glucose, and energy metabolism *via* FXR or TGR/M-BAR-mediated pathways. Furthermore, the clinical application of FXR and TGR/M-BAR agonists are ongoing.

Recent studies have focused on intestinal FXR signaling; however, conflicting data have been reported regarding the metabolic regulation of intestinal FXR activity. Further studies are necessary to determine intestinal FXR signaling taking into consideration various factors such as microbiota regulation, BA pool size, and BA composition.

## REFERENCES

- Hofmann AF, Borgstroem B. The Intraluminal Phase of Fat Digestion In Man: The Lipid Content OF The Micellar And Oil Phases of Intestinal Content Obtained During Fat Digestion and Absorption. *J Clin Invest* 1964; **43**: 247-257 [PMID: 14162533 DOI: 10.1172/jci104909]
- Urizar NL, Dowhan DH, Moore DD. The farnesoid X-activated receptor mediates bile acid activation of phospholipid transfer protein gene expression. *J Biol Chem* 2000; **275**: 39313-39317 [PMID: 10998425 DOI: 10.1074/jbc.M007998200]
- Maruyama T, Miyamoto Y, Nakamura T, Tamai Y, Okada H, Sugiyama E, Nakamura T, Itadani H, Tanaka K. Identification of membrane-type receptor for bile acids (M-BAR). *Biochem Biophys Res Commun* 2002; **298**: 714-719 [PMID: 12419312 DOI: 10.1016/S0006-291X(02)02550-0]
- Makishima M, Okamoto AY, Repa JJ, Tu H, Learned RM, Luk A, Hull MV, Lustig KD, Mangelsdorf DJ, Shan B. Identification of a nuclear receptor for bile acids. *Science* 1999; **284**: 1362-1365 [PMID: 10334992 DOI: 10.1126/science.284.5418.1362]
- Parks DJ, Blanchard SG, Bledsoe RK, Chandra G, Consler TG, Kliewer SA, Stimmel JB, Willson TM, Zavacki AM, Moore DD, Lehmann JM. Bile acids: natural ligands for an orphan nuclear receptor. *Science* 1999; **284**: 1365-1368 [PMID: 10334993 DOI: 10.1126/science.284.5418.1365]
- Forman BM, Goode E, Chen J, Oro AE, Bradley DJ, Perlmann T, Noonan DJ, Burka LT, McMorris T, Lamph WW, Evans RM, Weinberger C. Identification of a Nuclear Receptor That Is Activated by Farnesol Metabolites. *Cell* 1995; **81**: 687-693 [PMID: 7774010 DOI: 10.1016/0092-8674(95)90530-8]
- Watanabe M, Houten SM, Wang L, Moschetta A, Mangelsdorf DJ, Heyman RA, Moore DD, Auwerx J. Bile acids lower triglyceride levels via a pathway involving FXR, SHP, and SREBP-1c. *J Clin Invest* 2004; **113**: 1408-1418 [PMID: 15146238 DOI: 10.1172/JCI21025]
- Lee JM, Wagner M, Xiao R, Kim KH, Feng D, Lazar MA, Moore DD. Nutrient-sensing nuclear receptors coordinate autophagy. *Nature* 2014; **516**: 112-115 [PMID: 25383539 DOI: 10.1038/nature13961]
- Seok S, Fu T, Choi SE, Li Y, Zhu R, Kumar S, Sun X, Yoon G, Kang Y, Zhong W, Ma J, Kemper B, Kemper JK. Transcriptional regulation of autophagy by an FXR-CREB axis. *Nature* 2014; **516**: 108-111 [PMID: 25383523 DOI: 10.1038/nature13949]
- Settembre C, Ballabio A. Cell metabolism: autophagy transcribed. *Nature* 2014; **516**: 40-41 [PMID: 25383529 DOI: 10.1038/nature13939]
- Watanabe M, Houten SM, Matakai C, Christoffolete MA, Kim BW, Sato H, Messaddeq N, Harney JW, Ezaki O, Kodama T, Schoonjans K, Bianco AC, Auwerx J. Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. *Nature* 2006; **439**: 484-489 [PMID: 16400329 DOI: 10.1038/nature04330]
- Keitel V, Reinehr R, Gatsios P, Rupprecht C, Görg B, Selbach O, Häussinger D, Kubitz R. The G-protein coupled bile salt receptor TGR5 is expressed in liver sinusoidal endothelial cells. *Hepatology* 2007; **45**: 695-704 [PMID: 17326144 DOI: 10.1002/hep.21458]
- Ichikawa R, Takayama T, Yoneno K, Kamada N, Kitazume MT, Higuchi H, Matsuoka K, Watanabe M, Itoh H, Kanai T, Hisamatsu T, Hibi T. Bile acids induce monocyte differentiation toward interleukin-12 hypo-producing dendritic cells via a TGR5-dependent pathway. *Immunology* 2012; **136**: 153-162 [PMID: 22236403 DOI: 10.1111/j.1365-2567.2012.03554.x]
- Matsuzaki J, Suzuki H, Tsugawa H, Watanabe M, Hossain S, Arai E, Saito Y, Sekine S, Akaike T, Kanai Y, Mukaisho K, Auwerx J, Hibi T. Bile acids increase levels of microRNAs 221 and 222, leading to degradation of CDX2 during esophageal carcinogenesis. *Gastroenterology* 2013; **145**: 1300-1311 [PMID: 23933602 DOI: 10.1053/j.gastro.2013.08.008]
- Yang F, Huang X, Yi T, Yen Y, Moore DD, Huang W. Spontaneous development of liver tumors in the absence of the bile acid receptor farnesoid X receptor. *Cancer Res* 2007; **67**: 863-867 [PMID: 17283114 DOI: 10.1158/0008-5472.can-06-1078]
- Houten SM, Watanabe M, Auwerx J. Endocrine functions of bile acids. *EMBO J* 2006; **25**: 1419-1425 [PMID: 16541101 DOI: 10.1038/sj.emboj.7601049]
- Schwarz M, Russell DW, Dietschy JM, Turley SD. Alternate pathways of bile acid synthesis in the cholesterol 7 $\alpha$ -hydroxylase knockout mouse are not upregulated by either cholesterol or cholestyramine feeding. *J Lipid Res* 2001; **42**: 1594-1603 [PMID: 11590215]
- Russell DW, Setchell KD. Bile acid biosynthesis. *Biochemistry* 1992; **31**: 4737-4749 [PMID: 1591235 DOI: 10.1021/bi00135a001]
- Russell DW. The enzymes, regulation, and genetics of bile acid synthesis. *Annu Rev Biochem* 2003; **72**: 137-174 [PMID: 12543708 DOI: 10.1146/annurev.biochem.72.121801.161712]
- Amuro Y, Endo T, Higashino K, Uchida K, Yamamura Y. Serum, fecal and urinary bile acids in patients with mild and advanced liver cirrhosis. *Gastroenterol Jpn* 1981; **16**: 506-513 [PMID: 7327387 DOI: 10.1007/BF02774522]
- Sinal CJ, Tohkin M, Miyata M, Ward JM, Lambert G, Gonzalez FJ. Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis. *Cell* 2000; **102**: 731-744 [PMID: 11030617 DOI: 10.1016/S0092-8674(00)00062-3]
- Brendel C, Schoonjans K, Botrugno OA, Treuter E, Auwerx J. The small heterodimer partner interacts with the liver X receptor alpha and represses its transcriptional activity. *Mol Endocrinol* 2002; **16**: 2065-2076 [PMID: 12198243 DOI: 10.1210/me.2001-0194]
- Lu TT, Makishima M, Repa JJ, Schoonjans K, Kerr TA, Auwerx J, Mangelsdorf DJ. Molecular basis for feedback regulation of bile acid synthesis by nuclear receptors. *Mol Cell* 2000; **6**: 507-515 [PMID: 11030331 DOI: 10.1016/S1097-2765(00)00050-2]
- Holt JA, Luo G, Billin AN, Bisi J, McNeill YY, Kozarsky KF, Donahee M, Wang DY, Mansfield TA, Kliewer SA, Goodwin B, Jones SA. Definition of a novel growth factor-dependent signal cascade for the suppression of bile acid biosynthesis. *Genes Dev* 2003; **17**: 1581-1591 [PMID: 12815072 DOI: 10.1101/gad.1083503]
- Inagaki T, Choi M, Moschetta A, Peng L, Cummins CL, McDonald JG, Luo G, Jones SA, Goodwin B, Richardson JA, Gerard RD, Repa JJ, Mangelsdorf DJ, Kliewer SA. Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. *Cell Metab* 2005; **2**: 217-225 [PMID: 16213224 DOI: 10.1016/j.cmet.2005.09.001]
- Maruyama T, Tanaka K, Suzuki J, Miyoshi H, Harada N, Nakamura T, Miyamoto Y, Kanatani A, Tamai Y. Targeted disruption of G protein-coupled bile acid receptor 1 (Gpbar1/M-Bar) in mice. *J Endocrinol* 2006; **191**: 197-205 [PMID: 17065403 DOI: 10.1677/joe.1.06546]
- Vassileva G, Golovko A, Markowitz L, Abbondanzo SJ, Zeng M, Yang S, Hoos L, Tetzloff G, Levitan D, Murgolo NJ, Keane K, Davis HR, Hedrick J, Gustafson EL. Targeted deletion of Gpbar1 protects mice from cholesterol gallstone formation. *Biochem J* 2006; **398**: 423-430 [PMID: 16724960 DOI: 10.1042/bj20060537]
- Bouillon R, Carmeliet G, Lieben L, Watanabe M, Perino A, Auwerx J, Schoonjans K, Verstuyf A. Vitamin D and energy homeostasis: of mice and men. *Nat Rev Endocrinol* 2014; **10**: 79-87 [PMID: 24247221 DOI: 10.1038/nrendo.2013.226]
- Han S, Li T, Ellis E, Strom S, Chiang JY. A novel bile acid-

- activated vitamin D receptor signaling in human hepatocytes. *Mol Endocrinol* 2010; **24**: 1151-1164 [PMID: 20371703 DOI: 10.1210/me.2009-0482]
- 30 **Duran-Sandoval D**, Mautino G, Martin G, Percevault F, Barbier O, Fruchart JC, Kuipers F, Staels B. Glucose regulates the expression of the farnesoid X receptor in liver. *Diabetes* 2004; **53**: 890-898 [PMID: 15047603 DOI: 10.2337/diabetes.53.4.890]
  - 31 **Yamagata K**, Daitoku H, Shimamoto Y, Matsuzaki H, Hirota K, Ishida J, Fukamizu A. Bile acids regulate gluconeogenic gene expression via small heterodimer partner-mediated repression of hepatocyte nuclear factor 4 and Foxo1. *J Biol Chem* 2004; **279**: 23158-23165 [PMID: 15047713 DOI: 10.1074/jbc.M314322200]
  - 32 **Yamagata K**, Yoshimochi K, Daitoku H, Hirota K, Fukamizu A. Bile acid represses the peroxisome proliferator-activated receptor-gamma coactivator-1 promoter activity in a small heterodimer partner-dependent manner. *Int J Mol Med* 2007; **19**: 751-756 [PMID: 17390079 DOI: 10.3892/ijmm.19.5.751]
  - 33 **Ma K**, Saha PK, Chan L, Moore DD. Farnesoid X receptor is essential for normal glucose homeostasis. *J Clin Invest* 2006; **116**: 1102-1109 [PMID: 16557297 DOI: 10.1172/JCI25604]
  - 34 **Staybrook KR**, Bramlett KS, Savkur RS, Ficorilli J, Cook T, Christe ME, Michael LF, Burris TP. Regulation of carbohydrate metabolism by the farnesoid X receptor. *Endocrinology* 2005; **146**: 984-991 [PMID: 15564327 DOI: 10.1210/en.2004-0965]
  - 35 **Zhang Y**, Lee FY, Barrera G, Lee H, Vales C, Gonzalez FJ, Willson TM, Edwards PA. Activation of the nuclear receptor FXR improves hyperglycemia and hyperlipidemia in diabetic mice. *Proc Natl Acad Sci USA* 2006; **103**: 1006-1011 [PMID: 16410358 DOI: 10.1073/pnas.0506982103]
  - 36 **Watanabe M**, Horai Y, Houten SM, Morimoto K, Sugizaki T, Arita E, Matakai C, Sato H, Tanigawara Y, Schoonjans K, Itoh H, Auwerx J. Lowering bile acid pool size with a synthetic farnesoid X receptor (FXR) agonist induces obesity and diabetes through reduced energy expenditure. *J Biol Chem* 2011; **286**: 26913-26920 [PMID: 21632533 DOI: 10.1074/jbc.M111.248203]
  - 37 **Cariou B**, van Harmelen K, Duran-Sandoval D, van Dijk TH, Grefhorst A, Abdelkarim M, Caron S, Torpier G, Fruchart JC, Gonzalez FJ, Kuipers F, Staels B. The farnesoid X receptor modulates adiposity and peripheral insulin sensitivity in mice. *J Biol Chem* 2006; **281**: 11039-11049 [PMID: 16446356 DOI: 10.1074/jbc.M510258200]
  - 38 **Holst JJ**. The physiology of glucagon-like peptide 1. *Physiol Rev* 2007; **87**: 1409-1439 [PMID: 17928588 DOI: 10.1152/physrev.00034.2006]
  - 39 **Katsuma S**, Hirasawa A, Tsujimoto G. Bile acids promote glucagon-like peptide-1 secretion through TGR5 in a murine enteroendocrine cell line STC-1. *Biochem Biophys Res Commun* 2005; **329**: 386-390 [PMID: 15721318 DOI: 10.1016/j.bbrc.2005.01.139]
  - 40 **Pellicciari R**, Gioiello A, Macchiarulo A, Thomas C, Rosatelli E, Natalini B, Sardella R, Pruzanski M, Roda A, Pastorini E, Schoonjans K, Auwerx J. Discovery of 6alpha-ethyl-23(S)-methylcholic acid (S-EMCA, INT-777) as a potent and selective agonist for the TGR5 receptor, a novel target for diabetes. *J Med Chem* 2009; **52**: 7958-7961 [PMID: 20014870 DOI: 10.1021/jm901390p]
  - 41 **Thomas C**, Gioiello A, Noriega L, Strehle A, Oury J, Rizzo G, Macchiarulo A, Yamamoto H, Matakai C, Pruzanski M, Pellicciari R, Auwerx J, Schoonjans K. TGR5-mediated bile acid sensing controls glucose homeostasis. *Cell Metab* 2009; **10**: 167-177 [PMID: 19723493 DOI: 10.1016/j.cmet.2009.08.001]
  - 42 **Sato H**, Genet C, Strehle A, Thomas C, Lobstein A, Wagner A, Mioskowski C, Auwerx J, Saladin R. Anti-hyperglycemic activity of a TGR5 agonist isolated from *Olea europaea*. *Biochem Biophys Res Commun* 2007; **362**: 793-798 [PMID: 17825251 DOI: 10.1016/j.bbrc.2007.06.130]
  - 43 **Thomas C**, Auwerx J, Schoonjans K. Bile acids and the membrane bile acid receptor TGR5--connecting nutrition and metabolism. *Thyroid* 2008; **18**: 167-174 [PMID: 18279017 DOI: 10.1089/thy.2007.0255]
  - 44 **Müssig K**, Staiger H, Machicao F, Machann J, Schick F, Schäfer SA, Claussen CD, Holst JJ, Gallwitz B, Stefan N, Fritsche A, Häring HU. Preliminary report: genetic variation within the GPBAR1 gene is not associated with metabolic traits in white subjects at an increased risk for type 2 diabetes mellitus. *Metabolism* 2009; **58**: 1809-1811 [PMID: 19716570 DOI: 10.1016/j.metabol.2009.06.012]
  - 45 **Fromm H**, Eschler A, Töllner D, Canzler H, Schmidt FW. [In vivo dissolving of gall-stones: the effect of chenodeoxycholic acid. (author's transl)]. *Dtsch Med Wochenschr* 1975; **100**: 1619-1624 [PMID: 170051 DOI: 10.1055/s-0028-1106432]
  - 46 **Horton JD**, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest* 2002; **109**: 1125-1131 [PMID: 11994399 DOI: 10.1172/JCI15593]
  - 47 **Kast HR**, Nguyen CM, Sinal CJ, Jones SA, Laffitte BA, Reue K, Gonzalez FJ, Willson TM, Edwards PA. Farnesoid X-activated receptor induces apolipoprotein C-II transcription: a molecular mechanism linking plasma triglyceride levels to bile acids. *Mol Endocrinol* 2001; **15**: 1720-1728 [PMID: 11579204 DOI: 10.1210/mend.15.10.0712]
  - 48 **Ginsberg HN**, Le NA, Goldberg IJ, Gibson JC, Rubinstein A, Wang-Iverson P, Norum R, Brown WV. Apolipoprotein B metabolism in subjects with deficiency of apolipoproteins CIII and AI. Evidence that apolipoprotein CIII inhibits catabolism of triglyceride-rich lipoproteins by lipoprotein lipase in vivo. *J Clin Invest* 1986; **78**: 1287-1295 [PMID: 3095375 DOI: 10.1172/jci112713]
  - 49 **Claudel T**, Inoue Y, Barbier O, Duran-Sandoval D, Kosykh V, Fruchart J, Fruchart JC, Gonzalez FJ, Staels B. Farnesoid X receptor agonists suppress hepatic apolipoprotein CIII expression. *Gastroenterology* 2003; **125**: 544-555 [PMID: 12891557 DOI: 10.1016/S0016-5085(03)00896-5]
  - 50 **Inaba T**, Matsuda M, Shimamura M, Takei N, Terasaka N, Ando Y, Yasuno H, Koishi R, Makishima M, Shimomura I. Angiotensin-like protein 3 mediates hypertriglyceridemia induced by the liver X receptor. *J Biol Chem* 2003; **278**: 21344-21351 [PMID: 12672813 DOI: 10.1074/jbc.M213202200]
  - 51 **Sirvent A**, Claudel T, Martin G, Brozek J, Kosykh V, Dartail R, Hum DW, Fruchart JC, Staels B. The farnesoid X receptor induces very low density lipoprotein receptor gene expression. *FEBS Lett* 2004; **566**: 173-177 [PMID: 15147890 DOI: 10.1016/j.febslet.2004.04.026]
  - 52 **Hirokane H**, Nakahara M, Tachibana S, Shimizu M, Sato R. Bile acid reduces the secretion of very low density lipoprotein by repressing microsomal triglyceride transfer protein gene expression mediated by hepatocyte nuclear factor-4. *J Biol Chem* 2004; **279**: 45685-45692 [PMID: 15337761 DOI: 10.1074/jbc.M404255200]
  - 53 **Sinal CJ**, Yoon M, Gonzalez FJ. Antagonism of the actions of peroxisome proliferator-activated receptor-alpha by bile acids. *J Biol Chem* 2001; **276**: 47154-47162 [PMID: 11606578 DOI: 10.1074/jbc.M107000200]
  - 54 **Zhang Y**, Yin L, Anderson J, Ma H, Gonzalez FJ, Willson TM, Edwards PA. Identification of novel pathways that control farnesoid X receptor-mediated hypocholesterolemia. *J Biol Chem* 2010; **285**: 3035-3043 [PMID: 19996107 DOI: 10.1074/jbc.M109.083899]
  - 55 **Evans RM**, Barish GD, Wang YX. PPARs and the complex journey to obesity. *Nat Med* 2004; **10**: 355-361 [PMID: 15057233 DOI: 10.1038/nm1025]
  - 56 **Pineda Torra I**, Claudel T, Duval C, Kosykh V, Fruchart JC, Staels B. Bile acids induce the expression of the human peroxisome proliferator-activated receptor alpha gene via activation of the farnesoid X receptor. *Mol Endocrinol* 2003; **17**: 259-272 [PMID: 12554753 DOI: 10.1210/me.2002-0120]
  - 57 **Savkur RS**, Bramlett KS, Michael LF, Burris TP. Regulation of pyruvate dehydrogenase kinase expression by the farnesoid X receptor. *Biochem Biophys Res Commun* 2005; **329**: 391-396 [PMID: 15721319 DOI: 10.1016/j.bbrc.2005.01.141]
  - 58 **Pols TW**, Nomura M, Harach T, Lo Sasso G, Oosterveer MH, Thomas C, Rizzo G, Gioiello A, Adorini L, Pellicciari R, Auwerx J,



- Schoonjans K. TGR5 activation inhibits atherosclerosis by reducing macrophage inflammation and lipid loading. *Cell Metab* 2011; **14**: 747-757 [PMID: 22152303 DOI: 10.1016/j.cmet.2011.11.006]
- 59 **Wang YD**, Chen WD, Yu D, Forman BM, Huang W. The G-protein-coupled bile acid receptor, Gpbar1 (TGR5), negatively regulates hepatic inflammatory response through antagonizing nuclear factor  $\kappa$  light-chain enhancer of activated B cells (NF- $\kappa$ B) in mice. *Hepatology* 2011; **54**: 1421-1432 [PMID: 21735468 DOI: 10.1002/hep.24525]
- 60 **Kawamata Y**, Fujii R, Hosoya M, Harada M, Yoshida H, Miwa M, Fukusumi S, Habata Y, Itoh T, Shintani Y, Hinuma S, Fujisawa Y, Fujino M. A G protein-coupled receptor responsive to bile acids. *J Biol Chem* 2003; **278**: 9435-9440 [PMID: 12524422 DOI: 10.1074/jbc.M209706200]
- 61 **Sato H**, Macchiarulo A, Thomas C, Gioiello A, Une M, Hofmann AF, Saladin R, Schoonjans K, Pellicciari R, Auwerx J. Novel potent and selective bile acid derivatives as TGR5 agonists: biological screening, structure-activity relationships, and molecular modeling studies. *J Med Chem* 2008; **51**: 1831-1841 [PMID: 18307294 DOI: 10.1021/jm7015864]
- 62 **Bianco AC**, Silva JE. Nuclear 3,5,3'-triiodothyronine (T3) in brown adipose tissue: receptor occupancy and sources of T3 as determined by in vivo techniques. *Endocrinology* 1987; **120**: 55-62 [PMID: 3780570 DOI: 10.1210/endo-120-1-55]
- 63 **Cohade C**, Mourtzikos KA, Wahl RL. "USA-Fat": prevalence is related to ambient outdoor temperature-evaluation with 18F-FDG PET/CT. *J Nucl Med* 2003; **44**: 1267-1270 [PMID: 12902417]
- 64 **Hany TF**, Gharehpapagh E, Kamel EM, Buck A, Himms-Hagen J, von Schulthess GK. Brown adipose tissue: a factor to consider in symmetrical tracer uptake in the neck and upper chest region. *Eur J Nucl Med Mol Imaging* 2002; **29**: 1393-1398 [PMID: 12271425 DOI: 10.1007/s00259-002-0902-6]
- 65 **Saito M**. Brown adipose tissue as a regulator of energy expenditure and body fat in humans. *Diabetes Metab J* 2013; **37**: 22-29 [PMID: 23441053 DOI: 10.4093/dmj.2013.37.1.22]
- 66 **van Marken Lichtenbelt WD**, Vanhommerig JW, Smulders NM, Drossaerts JM, Kemerink GJ, Bouvy ND, Schrauwen P, Teule GJ. Cold-activated brown adipose tissue in healthy men. *N Engl J Med* 2009; **360**: 1500-1508 [PMID: 19357405 DOI: 10.1056/NEJMoa0808718]
- 67 **Cypess AM**, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, Kuo FC, Palmer EL, Tseng YH, Doria A, Kolodny GM, Kahn CR. Identification and importance of brown adipose tissue in adult humans. *N Engl J Med* 2009; **360**: 1509-1517 [PMID: 19357406 DOI: 10.1056/NEJMoa0810780]
- 68 **Virtanen KA**, Lidell ME, Orava J, Heglind M, Westergren R, Niemi T, Taittonen M, Laine J, Savisto NJ, Enerbäck S, Nuutila P. Functional brown adipose tissue in healthy adults. *N Engl J Med* 2009; **360**: 1518-1525 [PMID: 19357407 DOI: 10.1056/NEJMoa0808949]
- 69 **Seale P**, Bjork B, Yang W, Kajimura S, Chin S, Kuang S, Scimè A, Devarakonda S, Conroe HM, Erdjument-Bromage H, Tempst P, Rudnicki MA, Beier DR, Spiegelman BM. PRDM16 controls a brown fat/skeletal muscle switch. *Nature* 2008; **454**: 961-967 [PMID: 18719582 DOI: 10.1038/nature07182]
- 70 **Wu J**, Boström P, Sparks LM, Ye L, Choi JH, Giang AH, Khandekar M, Virtanen KA, Nuutila P, Schaart G, Huang K, Tu H, van Marken Lichtenbelt WD, Hoeks J, Enerbäck S, Schrauwen P, Spiegelman BM. Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. *Cell* 2012; **150**: 366-376 [PMID: 22796012 DOI: 10.1016/j.cell.2012.05.016]
- 71 **Tomlinson E**, Fu L, John L, Hultgren B, Huang X, Renz M, Stephan JP, Tsai SP, Powell-Braxton L, French D, Stewart TA. Transgenic mice expressing human fibroblast growth factor-19 display increased metabolic rate and decreased adiposity. *Endocrinology* 2002; **143**: 1741-1747 [PMID: 11956156 DOI: 10.1210/endo.143.5.8850]
- 72 **Abu-Elheiga L**, Matzuk MM, Kordari P, Oh W, Shaiknov G, Gu Z, Wakil SJ. Mutant mice lacking acetyl-CoA carboxylase 1 are embryonically lethal. *Proc Natl Acad Sci USA* 2005; **102**: 12011-12016 [PMID: 16103361 DOI: 10.1073/pnas.0505714102]
- 73 **Fu L**, John LM, Adams SH, Yu XX, Tomlinson E, Renz M, Williams PM, Soriano R, Corpuz R, Moffat B, Vandlen R, Simmons L, Foster J, Stephan JP, Tsai SP, Stewart TA. Fibroblast growth factor 19 increases metabolic rate and reverses dietary and leptin-deficient diabetes. *Endocrinology* 2004; **145**: 2594-2603 [PMID: 14976145 DOI: 10.1210/en.2003-1671]
- 74 **Fang S**, Suh JM, Reilly SM, Yu E, Osborn O, Lackey D, Yoshihara E, Perino A, Jacinto S, Lukasheva Y, Atkins AR, Khvat A, Schnabl B, Yu RT, Brenner DA, Coulter S, Liddle C, Schoonjans K, Olefsky JM, Saltiel AR, Downes M, Evans RM. Intestinal FXR agonism promotes adipose tissue browning and reduces obesity and insulin resistance. *Nat Med* 2015; **21**: 159-165 [PMID: 25559344 DOI: 10.1038/nm.3760]
- 75 **Ridlon JM**, Kang DJ, Hylemon PB. Bile salt biotransformations by human intestinal bacteria. *J Lipid Res* 2006; **47**: 241-259 [PMID: 16299351 DOI: 10.1194/jlr.R500013-JLR200]
- 76 **Sayin SI**, Wahlström A, Felin J, Jäntti S, Marschall HU, Bamberg K, Angelin B, Hyötyläinen T, Orešič M, Bäckhed F. Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. *Cell Metab* 2013; **17**: 225-235 [PMID: 23395169 DOI: 10.1016/j.cmet.2013.01.003]
- 77 **Li F**, Jiang C, Krausz KW, Li Y, Albert I, Hao H, Fabre KM, Mitchell JB, Patterson AD, Gonzalez FJ. Microbiome remodelling leads to inhibition of intestinal farnesoid X receptor signalling and decreased obesity. *Nat Commun* 2013; **4**: 2384 [PMID: 24064762 DOI: 10.1038/ncomms3384]
- 78 **Qi Y**, Jiang C, Cheng J, Krausz KW, Li T, Ferrell JM, Gonzalez FJ, Chiang JY. Bile acid signaling in lipid metabolism: metabolomic and lipidomic analysis of lipid and bile acid markers linked to anti-obesity and anti-diabetes in mice. *Biochim Biophys Acta* 2015; **1851**: 19-29 [PMID: 24796972 DOI: 10.1016/j.bbalip.2014.04.008]
- 79 **Jiang C**, Xie C, Li F, Zhang L, Nichols RG, Krausz KW, Cai J, Qi Y, Fang ZZ, Takahashi S, Tanaka N, Desai D, Amin SG, Albert I, Patterson AD, Gonzalez FJ. Intestinal farnesoid X receptor signaling promotes nonalcoholic fatty liver disease. *J Clin Invest* 2015; **125**: 386-402 [PMID: 25500885 DOI: 10.1172/jci76738]
- 80 **Trabelsi MS**, Daoudi M, Prawitt J, Ducastel S, Touche V, Sayin SI, Perino A, Brighton CA, Sebt Y, Kluza J, Briand O, Dehondt H, Vallez E, Dorchies E, Baud G, Spinelli V, Hennuyer N, Caron S, Bantubungi K, Caiazzo R, Reimann F, Marchetti P, Lefebvre P, Bäckhed F, Gribble FM, Schoonjans K, Pattou F, Tailleux A, Staels B, Lestavel S. Farnesoid X receptor inhibits glucagon-like peptide-1 production by enteroendocrine L cells. *Nat Commun* 2015; **6**: 7629 [PMID: 26134028 DOI: 10.1038/ncomms8629]
- 81 **Nakatani H**, Kasama K, Oshiro T, Watanabe M, Hirose H, Itoh H. Serum bile acid along with plasma incretins and serum high-molecular weight adiponectin levels are increased after bariatric surgery. *Metabolism* 2009; **58**: 1400-1407 [PMID: 19570554 DOI: 10.1016/j.metabol.2009.05.006]
- 82 **Zhang H**, DiBaise JK, Zuccolo A, Kudrna D, Braidotti M, Yu Y, Parameswaran P, Crowell MD, Wing R, Rittmann BE, Krajmalnik-Brown R. Human gut microbiota in obesity and after gastric bypass. *Proc Natl Acad Sci USA* 2009; **106**: 2365-2370 [PMID: 19164560 DOI: 10.1073/pnas.0812600106]
- 83 **Ryan KK**, Tremaroli V, Clemmensen C, Kovatcheva-Datchary P, Myronovych A, Karns R, Wilson-Pérez HE, Sandoval DA, Kohli R, Bäckhed F, Seeley RJ. FXR is a molecular target for the effects of vertical sleeve gastrectomy. *Nature* 2014; **509**: 183-188 [PMID: 24670636 DOI: 10.1038/nature13135]
- 84 **Watanabe M**, Morimoto K, Houten SM, Kaneko-Iwasaki N, Sugizaki T, Horai Y, Mataka C, Sato H, Murahashi K, Arita E, Schoonjans K, Suzuki T, Itoh H, Auwerx J. Bile acid binding resin improves metabolic control through the induction of energy expenditure. *PLoS One* 2012; **7**: e38286 [PMID: 22952571 DOI: 10.1371/journal.pone.0038286PONE-D-11-16446]
- 85 **Shang Q**, Saumoy M, Holst JJ, Salen G, Xu G. Colesevelam improves insulin resistance in a diet-induced obesity (F-DIO) rat model by increasing the release of GLP-1. *Am J Physiol Gastrointest Liver Physiol* 2010; **298**: G419-G424 [PMID: 20044510 DOI: 10.1152/ajpgi.00000.2010]



- 10.1152/ajpgi.00362.2009]
- 86 **Chen L**, McNulty J, Anderson D, Liu Y, Nystrom C, Bullard S, Collins J, Handlon AL, Klein R, Grimes A, Murray D, Brown R, Krull D, Benson B, Kleymenova E, Remlinger K, Young A, Yao X. Cholestyramine reverses hyperglycemia and enhances glucose-stimulated glucagon-like peptide 1 release in Zucker diabetic fatty rats. *J Pharmacol Exp Ther* 2010; **334**: 164-170 [PMID: 20413600 DOI: 10.1124/jpet.110.166892]
- 87 **Garg A**, Grundy SM. Cholestyramine therapy for dyslipidemia in non-insulin-dependent diabetes mellitus. A short-term, double-blind, crossover trial. *Ann Intern Med* 1994; **121**: 416-422 [PMID: 8053615 DOI: 10.7326/0003-4819-121-6-199409150-00004]
- 88 **Suzuki T**, Oba K, Igari Y, Matsumura N, Watanabe K, Futami-Suda S, Yasuoka H, Ouchi M, Suzuki K, Kigawa Y, Nakano H. Colestimide lowers plasma glucose levels and increases plasma glucagon-like PEPTIDE-1 (7-36) levels in patients with type 2 diabetes mellitus complicated by hypercholesterolemia. *J Nippon Med Sch* 2007; **74**: 338-343 [PMID: 17965527 DOI: 10.1272/jnms.74.338]
- 89 **Zieve FJ**, Kalin MF, Schwartz SL, Jones MR, Bailey WL. Results of the glucose-lowering effect of WelChol study (GLOWS): a randomized, double-blind, placebo-controlled pilot study evaluating the effect of colestevlam hydrochloride on glycemic control in subjects with type 2 diabetes. *Clin Ther* 2007; **29**: 74-83 [PMID: 17379048 DOI: 10.1016/j.clinthera.2007.01.003]
- 90 **Bays HE**, Goldberg RB, Truitt KE, Jones MR. Colesevelam hydrochloride therapy in patients with type 2 diabetes mellitus treated with metformin: glucose and lipid effects. *Arch Intern Med* 2008; **168**: 1975-1983 [PMID: 18852398 DOI: 10.1001/archinte.168.18.1975]
- 91 **Fonseca VA**, Rosenstock J, Wang AC, Truitt KE, Jones MR. Colesevelam HCl improves glycemic control and reduces LDL cholesterol in patients with inadequately controlled type 2 diabetes on sulfonylurea-based therapy. *Diabetes Care* 2008; **31**: 1479-1484 [PMID: 18458145 DOI: 10.2337/dc08-0283]
- 92 **Goldberg RB**, Fonseca VA, Truitt KE, Jones MR. Efficacy and safety of colestevlam in patients with type 2 diabetes mellitus and inadequate glycemic control receiving insulin-based therapy. *Arch Intern Med* 2008; **168**: 1531-1540 [PMID: 18663165 DOI: 10.1001/archinte.168.14.1531]
- 93 **Tagawa H**, Irie J, Itoh A, Kusumoto Y, Kato M, Kobayashi N, Tanaka K, Morinaga R, Fujita M, Nakajima Y, Morimoto K, Sugizaki T, Kawano Y, Yamada S, Kawai T, Watanabe M, Itoh H. Bile acid binding resin improves hepatic insulin sensitivity by reducing cholesterol but not triglyceride levels in the liver. *Diabetes Res Clin Pract* 2015; **109**: 85-94 [PMID: 25981325 DOI: 10.1016/j.diabres.2015.04.025]
- 94 **Henry RR**, Aroda VR, Mudaliar S, Garvey WT, Chou HS, Jones MR. Effects of colestevlam on glucose absorption and hepatic/peripheral insulin sensitivity in patients with type 2 diabetes mellitus. *Diabetes Obes Metab* 2012; **14**: 40-46 [PMID: 21831167 DOI: 10.1111/j.1463-1326.2011.01486.x]
- 95 **Porez G**, Prawitt J, Gross B, Staels B. Bile acid receptors as targets for the treatment of dyslipidemia and cardiovascular disease. *J Lipid Res* 2012; **53**: 1723-1737 [PMID: 22550135 DOI: 10.1194/jlr.R024794]
- 96 **Pellicciari R**, Fiorucci S, Camaioni E, Clerici C, Costantino G, Maloney PR, Morelli A, Parks DJ, Willson TM. 6alpha-ethylchenodeoxycholic acid (6-ECDCA), a potent and selective FXR agonist endowed with anticholestatic activity. *J Med Chem* 2002; **45**: 3569-3572 [PMID: 12166927 DOI: 10.1021/jm025529g]
- 97 **Fiorucci S**, Antonelli E, Rizzo G, Renga B, Mencarelli A, Riccardi L, Orlandi S, Pellicciari R, Morelli A. The nuclear receptor SHP mediates inhibition of hepatic stellate cells by FXR and protects against liver fibrosis. *Gastroenterology* 2004; **127**: 1497-1512 [PMID: 15521018 DOI: 10.1053/j.gastro.2004.08.001]
- 98 **Fiorucci S**, Clerici C, Antonelli E, Orlandi S, Goodwin B, Sadeghpour BM, Sabatino G, Russo G, Castellani D, Willson TM, Pruzanski M, Pellicciari R, Morelli A. Protective effects of 6-ethylchenodeoxycholic acid, a farnesoid X receptor ligand, in estrogen-induced cholestasis. *J Pharmacol Exp Ther* 2005; **313**: 604-612 [PMID: 15644430 DOI: 10.1124/jpet.104.079665]
- 99 **Li T**, Holmstrom SR, Kir S, Umetani M, Schmidt DR, Klierwer SA, Mangelsdorf DJ. The G protein-coupled bile acid receptor, TGR5, stimulates gallbladder filling. *Mol Endocrinol* 2011; **25**: 1066-1071 [PMID: 21454404 DOI: 10.1210/me.2010-0460]

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Basic Study

## Preliminary study on overproduction of reactive oxygen species by neutrophils in diabetes mellitus

Noridzzaida Ridzuan, Cini Mathew John, Pratheep Sandrasaigaran, Maryam Maqbool, Lee Chuen Liew, Jonathan Lim, Rajesh Ramasamy

Noridzzaida Ridzuan, Cini Mathew John, Pratheep Sandrasaigaran, Maryam Maqbool, Lee Chuen Liew, Rajesh Ramasamy, Immunology Laboratory, Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia

Noridzzaida Ridzuan, Maryam Maqbool, Rajesh Ramasamy, Stem Cell Research Laboratory, Genetic and Regenerative Medicine Research Center, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia

Cini Mathew John, Department of Physiology and Pharmacology, Faculty of Medicine, University of Calgary, Calgary, AB T2N 4N1, Canada

Jonathan Lim, Department of Medicine, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia

**Author contributions:** All the authors contributed to this manuscript.

**Institutional review board statement:** No institutional review board statement because this is a preliminary study conducted with a very limited number of patients based on their voluntary contribution and thus ethical approval from institute was not sought. However, the handling of samples was carried out in accordance with ethical values and standard procedures.

**Informed consent statement:** Informed consent was obtained from the voluntarily recruited patients. They were briefed on the purpose of the study and its implication prior to donating 10 mL of peripheral blood. History of medication and management of diabetes was collected with permission of the relevant patients.

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**Correspondence to:** Rajesh Ramasamy, PhD, Immunology Laboratory, Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang Hospital Street, Serdang 43400, Selangor, Malaysia. [rajesh@upm.edu.my](mailto:rajesh@upm.edu.my)  
Telephone: +603-89-472377  
Fax: +603-89-412787

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### Abstract

**AIM:** To assess the amount and pattern of reactive oxygen species (ROS) production in diabetic patient-derived neutrophils.

**METHODS:** Blood samples from type 2 diabetes mellitus (DM) patients and volunteers (controls) were subjected to neutrophil isolation and the assessment of neutrophil oxidative burst using chemiluminescence assay. Neutrophils were activated by using phorbol myristate acetate (PMA) and neutrophils without activation were kept as a negative control. The chemilu-

minescence readings were obtained by transferring cell suspension into a 1.5 mL Eppendorf tube, with PMA and luminol. Reaction mixtures were gently vortexed and placed inside luminometer for a duration of 5 min.

**RESULTS:** Our results showed that in the resting condition, the secretion of ROS in normal non-diabetic individuals was relatively low compared to diabetic patients. However, the time scale observation revealed that the secreted ROS declined accordingly with time in non-diabetic individuals, yet such a reduction was not detected in diabetic patients where at all the time points, the secretion of ROS was maintained at similar magnitudes. This preliminary study demonstrated that ROS production was significantly higher in patients with DM compared to non-diabetic subjects in both resting and activated conditions.

**CONCLUSION:** The respiratory burst activity of neutrophils could be affected by DM and the elevation of ROS production might be an aggravating factor in diabetic-related complications.

**Key words:** Neutrophils; Diabetes mellitus; Reactive oxygen species; Chemiluminescence

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**Core tip:** This is a preliminary study that investigates the activation status of peripheral blood-derived neutrophils in type 2 diabetes. This study clearly indicated that the neutrophils from type 2 diabetic patients are highly activated upon *in vitro* stimulation and hence produce greater amounts of reactive oxygen species (ROS) compared to a normal individual. Release of a greater volume of ROS could serve as an additional risk for end organ injury in type 2 diabetes mellitus.

Ridzuan N, John CM, Sandrasaigaran P, Maqbool M, Liew LC, Lim J, Ramasamy R. Preliminary study on overproduction of reactive oxygen species by neutrophils in diabetes mellitus. *World J Diabetes* 2016; 7(13): 271-278 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v7/i13/271.htm> DOI: <http://dx.doi.org/10.4239/wjd.v7.i13.271>

## INTRODUCTION

Neutrophils are a crucial first line cellular host defence against infections as they are potent mediators of inflammation<sup>[1]</sup>. Elimination of pathogens by neutrophils follows a sequence of events such as adherence, chemotaxis, phagocytosis, microbial killing and apoptosis. The microbial killing by the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) *via* activation of respiratory burst cascades plays a vital role in removing phagocytosed microbes<sup>[2]</sup>. ROS and RNS produced during the respiratory burst provide an important neutrophil-

mediated defence system, yet the overproduction ROS can trigger vascular damage in chronic diseases such as hypertension and diabetes mellitus (DM)<sup>[3]</sup>.

In recent years, the understanding of DM has changed from the perception of a chronic metabolic disease to an immune-mediated disease. Many expert reports advocate that DM may be a paradox of immune reactivity, which results in the development of the diabetic state and may lead to severe complications. The alteration of an innate immune response due to hyperglycemia has been recognised as the major factor resulting in the development of DM. Both type 1 and 2 DM are afflicted by the host immune system. Autoimmune T cells and autoantibody production against pancreatic beta cells are responsible for the development of type 1 DM, whereas the chronic low-grade inflammation and activation of the innate immune system are closely related to the pathogenesis of type 2 DM<sup>[4]</sup>. The elevated levels of inflammatory markers such as tumor necrosis factor- $\alpha$ , interleukin-6 and C-reactive protein were seen in subjects with diabetes<sup>[5]</sup>.

In diabetic patients, ROS is produced *via* glucose autooxidation and non-enzymatic protein glycation in various tissues such as neural cells, lens crystalline and recently reported in pancreatic  $\beta$ -cells<sup>[4,6]</sup>. Since the activity of antioxidant enzymes in the pancreas is relatively low compared to other tissues, the pancreas is one of the organs sensitive to an oxygen radical attack<sup>[7]</sup>. Apart from that, the uncontrolled release of free radical nitric oxide (NO) from endothelium also possesses a toxic effect on microvasculature<sup>[8,9]</sup>. The excessive and ill-controlled NO and ROS can react to form peroxy-nitrite anion, a highly reactive and toxic compound which rapidly decomposes to hydroxyl anion and nitrogen dioxide<sup>[10]</sup>. This possibly leads to cytotoxic and cytostatic effects on parenchymal cells and heads to irreversible pathologies<sup>[11,12]</sup>. Overall, ROS formation is considered a direct consequence of hyperglycemia. Hence the glycation process and the subsequent oxidative stress pave a way to the detrimental effects of DM<sup>[13]</sup>.

The prime effector function of a neutrophil relies on its ability to generate ROS within the phagolysosome for the degradation of engulfed pathogens. However, the excessive or inappropriate production of these reactive compounds may lead to detrimental effects such as hypertension, atherosclerosis and DM. The elevated oxidative stress which results from superoxide release by neutrophils in the diabetic condition is well documented<sup>[14-17]</sup>. The assessment of neutrophil-mediated respiratory burst activity from Hispanic diabetic individuals demonstrated a significant rise in ROS outburst compared to the normal group. Interestingly, upon treatment with PKC inhibitors and azithromycin, the magnitude of the respiratory burst response was substantially reduced<sup>[14]</sup>. Similarly, the high levels of glucose and AGE also induced neutrophil activation and subsequently escalated the oxidative stress *via* the RAGE-ERK1/2 pathway<sup>[15]</sup>. It is clear that the harmful effect of ROS is

**Table 1** Demographics and characteristics of the patients with type 2 diabetes mellitus

Patient	Age	Duration (yr)	HbA1c (%)	Family history	Types of medication
1	65	12	6.2	Father and mother	Metformin, atorvastatin and multivitamins
2	72	17	6.6	Mother	Glibenclamide, insulin (humapen), ecospirin and viatril S
3	82	28	6.1	-	Metformin, insulin (mixtard), cardipirin, prostin and lovastatin
4	69	11	6.3	Father	Diamicron MR, simvastatin
5	65	13	6.2	Mother	Herbal medication, glibenclamide and metoprolol
6	64	14	7.2	Father	Insulin, metformin, simvastatin and captopril

very much linked with the augmented production of the advanced glycated end-products (AGE) and their cognate receptors (RAGE). The ligation between AGE and RAGE potentially increases the cytosolic ROS and facilitates mitochondrial superoxide production in the hyperglycemic condition<sup>[18]</sup>. Although the actual mechanism that governs the production and release of ROS in diabetic patients' neutrophils is still elusive, it does not negate the possible role of damaged mitochondria to generate an excess amount of superoxide which is fuelled by a sustained supply of NADH<sup>[19]</sup>.

Despite growing data which show the role of oxidative stress in the etiology and pathophysiology of DM, there are no consistent results of ROS overproduction in diabetic patients<sup>[4,6-9]</sup>. Therefore, the current pilot study was designed to assess the pattern of ROS production in type 2 DM. The outcome of this present study showed that neutrophils from patients with DM constitutively secrete a significantly higher volume of ROS in both resting and activated conditions.

## MATERIALS AND METHODS

### Subjects

This is an experimental study where a total of 6 type 2 DM patients (duration of disease 11-28 years) aged 64-82 years were included in this study. The patients and normal subjects were voluntarily recruited, briefed on the purpose of the study and verbal consent was obtained. Patients were selected based on the inclusion criteria of type 2 DM for more than 10 years duration and the age range of 60-82 years. The exclusion criteria were patients undergoing dialysis, inflicted with anemia, polycythemia and gout or had a history of severe immunological, hepatic, cardiac, renal, hematological or other organ impairment. The details of HbA1c and anti-diabetic treatment of each patient were extracted from the latest laboratory screening. The particulars of the patients are shown in Table 1. Samples of the non-diabetic control group were obtained from 3 volunteers aged between 30-50 years old.

### Blood sampling

Ten millilitres of a peripheral venous blood sample from diabetic patients and non-diabetic individuals were collected by a certified phlebotomist. Whole blood was collected in two 9 mL vacutainers with sodium heparin as anticoagulant (Greiner bio-one, Australia). Peripheral

blood samples were processed immediately after the collection.

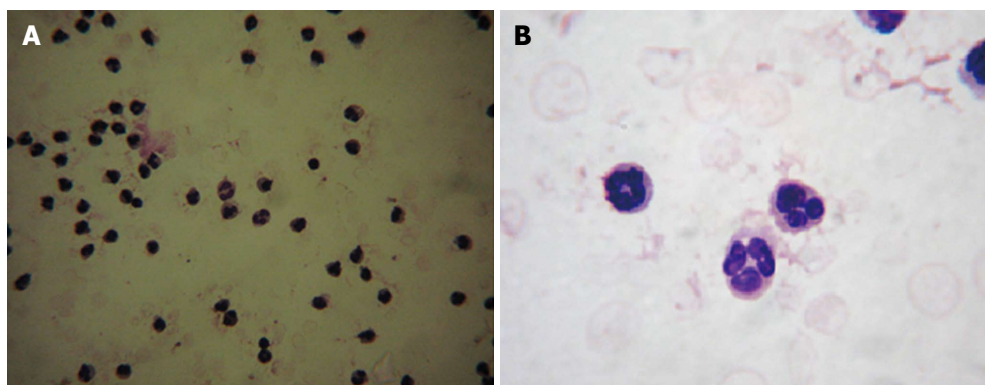
### Neutrophil isolation

Neutrophil isolation and verification by morphology were conducted as per our established laboratory procedures<sup>[3,20]</sup>. Briefly, 10 mL of peripheral blood was collected and diluted in 1 × Hank's balanced salt solution (HBSS Gibco, United Kingdom) medium at 1:1 ratio. Ten millilitres (10 mL) diluted blood was then layered over 5 mL Ficoll-Paque solution (GE Health care, life sciences, Sweden) and centrifuged at 1800 rpm for 30 min at room temperature. Once the unwanted mononuclear cells and plasma were decanted, the red cell pellet which contains the polymorphonuclear cells (PMN) was suspended in 5 mL HBSS, layered on 3% Dextran (Fisher Scientific, NJ, United States) and sedimented at room temperature for 45-60 min. The sedimented supernatant was further subjected to the RBC lysing procedure to obtain uncontaminated PMN. Leishman staining was performed to confirm the neutrophil morphology. Briefly, a few drops of the neutrophil suspension were spread on a glass slide and covered with Leishman solution (Merck, Germany) for 1 min. Subsequently, the smear was immersed in phosphate buffer solution for 15 min. The slide was rinsed off with tap water, dried and examined under the light microscope at 20 × and 40 × magnifications. The viability of cells was determined by trypan blue exclusion during the manual cell counting process.

### Assessment of neutrophil oxidative burst

Oxidative burst by human neutrophils was measured by production of ROS through chemiluminescence assay. Freshly isolated neutrophils ( $0.5 \times 10^5$  cells/well) were seeded in complete Roswell Park Memorial Institute medium without phenol red (Gibco, United Kingdom) and were concurrently activated with 500 nmol/L phorbol myristate acetate (PMA) (Sigma, Germany). Neutrophils without activation were kept as a negative control. Cells and the tested compound were maintained at 37 °C in a water bath prior to adding the stimulants. Readings were obtained by transferring the cell suspension into a 1.5 mL Eppendorf tube, with 100 µL PMA and 100 µL luminol (500 µmol/L). Reaction mixtures containing a final volume of 1000 µL were gently vortexed and placed inside Glomax luminometer 20/20 (Promega) for a duration of 5 min.





**Figure 1 Morphological analysis of isolated neutrophils.** Isolated neutrophils were smeared on a glass slide and labeled with Leishman stain. Under the 20 × magnification, cells appeared homogeneous (A) and 40 × magnification exhibited the multi-lobulated nucleus (B).

### Statistical analysis

Data are expressed as mean ± SD<sup>[21]</sup>. Differences were considered significant at  $P \leq 0.001$ . Statistical analyses were conducted using 2-way ANOVA using Microsoft Office 2007 (Excel).

## RESULTS

### Isolation and confirmation of neutrophils

Employing density gradient centrifugation followed by a dextran sedimentation procedure, peripheral blood from non-diabetic individuals and diabetic patients were fractionated into several layers. The layer enriched with neutrophils was further subjected to the hypotonic red blood lysis to obtain a pure population of neutrophils. Isolated neutrophils were labeled with Leishman staining to confirm the purity of neutrophils. The isolation process yielded more than 85% of the pure neutrophil population. Examination under the light microscope with low magnification revealed that isolated cells were uniform and free from RBC and mononuclear cell contamination (Figure 1A). The morphology of neutrophils was further confirmed by microscopical examination with higher magnification where cells displaying a 3-5 lobulated nucleus were confirmed as neutrophils (Figure 1B).

### Neutrophils from diabetic patients constitutively produced a higher amount of ROS in a resting condition

In a resting condition, both neutrophils that were isolated from non-diabetic individuals and diabetic patients showed a basal amount of ROS production. It was noticed that neutrophils from non-diabetic individuals secreted ROS approximately 8000 chemiluminescence counts (CC) at the initial 10 s and the secretion declined with time when the lowest 2000 CC was noticed at 50 s. However, neutrophils from diabetic patients exhibited a statistically significant increase in ROS secretion (13000-14000 CC) ( $P \leq 0.001$ ) and the amount was maintained throughout the measurement points up to 50 s (Figure 2). In order to determine whether a similar ROS production pattern could be observed when stimulated, a potent microbial agent, PMA was used to induce

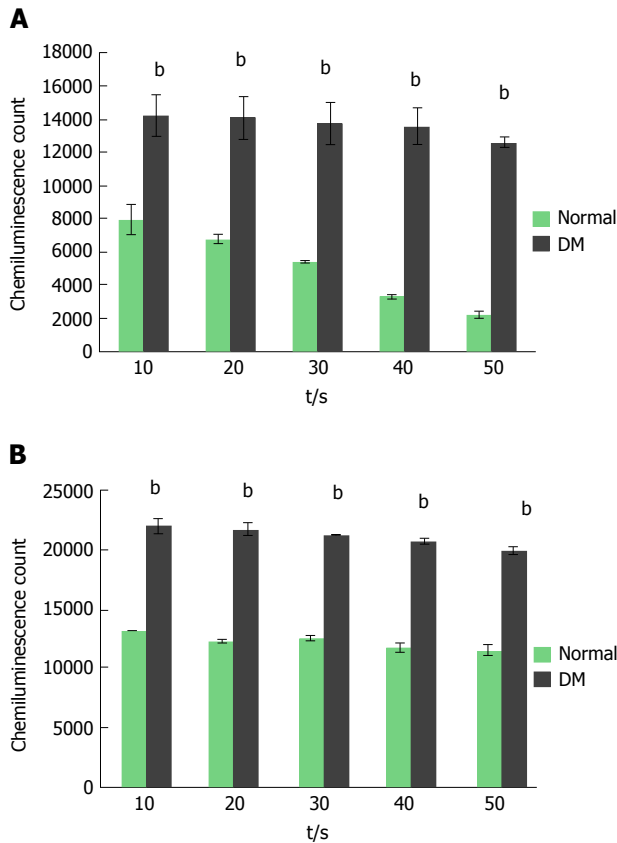
the neutrophil's ROS secretion. As expected, PMA profoundly increased ROS secretion at all measured time points regardless of the source of neutrophils. The secretion of ROS from neutrophils in non-diabetic and diabetic subjects was elevated at approximately 13000 CC and 21000 CC, respectively. The amount of ROS secreted from activated neutrophils of non-diabetic and diabetic subjects was maintained throughout the measurement points. Besides that, neutrophils from both non-diabetic and diabetic subjects displayed a maximal amount of secretion at the initial 10 s of the measurement period.

### Neutrophils from diabetic patients secreted higher ROS in both resting and activated conditions compared to the non-diabetic subjects

The maximal production of ROS by neutrophils of non-diabetic and diabetic subjects during any time point of the measured period was recorded. In a resting condition, the maximal amount of ROS produced by neutrophils of non-diabetic and diabetic subjects were 5000 CC and 13000 CC, respectively, whereas, PMA activated neutrophils of non-diabetic and diabetic subjects secreted 14000 CC and 20000 CC, respectively. In both resting and activated conditions, neutrophils from diabetic patients showed a statistically significant elevation of ROS production. However, a radical escalation index of ROS was noticed in the resting condition where ROS secreted from neutrophils of diabetic patients was approximately 2.8 fold higher than non-diabetic controls (Figure 3).

## DISCUSSION

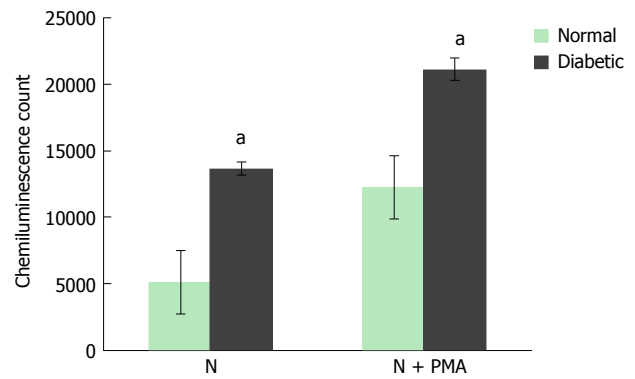
Neutrophils play a major role in innate immunity by executing acute inflammation due to infectious agents. Unlike other immune cells, neutrophils exist in peripheral blood in a larger quantity and the loss of neutrophils due to inflammation is rapidly substituted by its production in bone marrow. Due to its important role in the early inflammatory process, neutrophils are able to migrate towards the site of inflammation through the tight epithelial junction. The most critical effector functions of



**Figure 2 Temporal analysis of reactive oxygen species secreted by neutrophils of non-diabetic and diabetic subjects.** Isolated neutrophils were measured for ROS secretion via chemiluminescence method at every 10 s over a period of 50 s. Neutrophils isolated from diabetic patients showed a higher and consistent secretion of ROS in resting condition (A). PMA stimulation elevated the ROS secretion from neutrophils of both non-diabetic and diabetic subjects. Neutrophils from diabetic patients produced a higher amount of ROS compared to non-diabetic subjects. ROS production was determined at every 10 s beginning from the administration of PMA to the time point where the ROS production reached a maximal level and started to decline (B). Data expressed as mean  $\pm$  SD and the statistical significance was determined at  $^bP$  value  $\leq 0.001$ . ROS: Reactive oxygen species; PMA: Phorbol myristate acetate.

neutrophils are phagocytosis and killing the invading bacteria through the activation of the respiratory burst. Respiratory or oxidative burst in neutrophils starts with a consequent formation of ROS, such as superoxide radicals ( $O_2^-$ ) and hydrogen peroxide ( $H_2O_2$ ), and RNS, such as NO and peroxynitrate anion ( $ONOO^-$ )<sup>[22]</sup>. Besides inflammation induced by pathogens, neutrophils can be activated to produce free oxygen radicals and other superoxide derivatives with a variety of stimuli, such as the chemotactic peptide N-formyl methyl leucyl phenylalanine, the anaphylatoxin C5a, platelet-activating factor, leukotriene B, PMA and calcium ionophores<sup>[23-29]</sup>.

Oxidative stress resulting from a raised ROS level has become a common reflection of many chronic illnesses<sup>[3]</sup>. The present preliminary study showed that neutrophils from type 2 DM produced a significantly higher amount of ROS compared to non-diabetic individuals in both resting and activated conditions. The current result is in line with Houstis *et al.*<sup>[30]</sup> (2006) who demonstrated the involvement of increased ROS production in insulin



**Figure 3 Total reactive oxygen species produced by neutrophils of non-diabetic and diabetic subjects.** Neutrophils isolated from non-diabetic individuals and diabetic patients subjected for ROS secretion at resting condition and upon stimulation with PMA. The maximal production of ROS at any time point over a period of 50 s was recorded and compared. Neutrophils from diabetic patients showed a higher production of ROS at resting and activated conditions. Data expressed as mean  $\pm$  SD and the statistical significance was determined at  $^aP$  value  $\leq 0.05$ . ROS: Reactive oxygen species; PMA: Phorbol myristate acetate.

resistance in type 2 DM using a cell culture model and murine models. Conversely, Alba-Loureiro *et al.*<sup>[31]</sup> (2007) reported the secretion of ROS in DM was indeed reduced compared with normal controls. This observation was based on the assessment of neutrophil activities, such as chemotaxis, phagocytosis, ROS production and microbial killing, where these activities consume a substantial amount of ATP. Since diabetes affects energy metabolism, it could also result in down-regulation or a decrease in neutrophil activities. The pattern of ROS production in patients with DM is not consistent and may be due to many reasons. The inclusion of patients undergoing a specific regime of drugs that lower the overall oxidative stress might have confounded the experimental outcomes. Besides that, utilization of different techniques to isolate neutrophils and detect ROS might possibly affect the final volume of ROS secreted. The laboratory procedures such as hypotonic lysis of RBC for isolating neutrophils from peripheral blood and the long duration of sample processing may potentially affect the viability of neutrophils, thus reflected as reduced ROS secretion. In the current study, the isolation of neutrophils was conducted within 2-3 h of peripheral blood withdrawal and a chemiluminescence technique was utilised for detecting ROS over a period of time. This technique is highly sensitive as it utilizes luminol which allows detection of intracellular and extracellular ROS and RNS, such as  $O_2^-$ ,  $H_2O_2$ , hydroxyl radical (HO), hypochlorous acid (HOCl), NO and  $ONOO^-$ . Furthermore, morphological observation of isolated neutrophils displayed a healthier appearance and high viability.

This present study also revealed that as the time increased during ROS measurement over a total of 50 s, the ROS production in neutrophils of resting non-diabetic subjects constantly reduced, whereas the level of ROS from neutrophils of diabetic patients was maintained throughout the measurement period. The constant

and elevated secretion of ROS at all the time points by neutrophils of diabetic patients could be a consequence of the raised oxidative burst in DM. However, this could be a serious phenomenon where neutrophils of diabetic patients may have lost the ability to switch off or tame down the respiratory burst activity, hence predisposing the patients to microvascular injuries. The continuous suboptimal activation of the respiratory burst in neutrophils of diabetic patients may also deplete ROS or exhaust the mechanism that generates ROS, rendering neutrophils inefficient and thus increasing susceptibility to microbial infection. This could also be a potential cause for slower wound healing and occurrence of gangrene in diabetic patients. When neutrophils were stimulated with a robust infectious agent-derived substance, PMA, neutrophils of both non-diabetic and diabetic subjects secreted a tremendous amount of ROS. Our results demonstrated that neutrophils responded well to PMA stimulation, as reported by Ramasamy *et al.*<sup>[3]</sup> in 2010. Although the current study did not decipher the potential signaling pathway that might be involved in PMA stimulation, human neutrophils showed that the activation of the protein kinase C (PKC) signaling cascade serves as an inducer of rapid ROS synthesis. Notably, the 2.8 fold increase of secreted ROS between non-diabetic and diabetic subjects in a resting condition was much higher compared to the induced activated condition. This supports our notion that neutrophils from diabetic patients are in a state of auto ROS secretion, which explains the possible contribution to microvascular injuries in DM.

The overproduction of ROS by the neutrophil-mediated respiratory burst can be controlled either *via* inhibition of ROS-generating enzymes, NADPH oxidase or through the direct ROS-scavenging effect. In the physiological condition, the activation of NADPH oxidase in generating ROS in neutrophils is beneficial for host defence. In this case, overproduction of free radicals and proteolytic enzymes used as defences against infections can be highly toxic to the surrounding cells and tissues<sup>[32]</sup>. Nevertheless, it can be deleterious to the host if the enzyme cascade is inappropriately activated or loses its control. Hence, drugs such pyrazolones and its derivatives such as aminopyrine and dipyrone can be used in the management of DM. The study conducted by Costa *et al.*<sup>[32]</sup> (2006) showed that these drugs not only normalize the glucose level but scavenge the over-produced neutrophil reactive species. Besides that, plasma glucose level should also be strictly controlled as hyperglycemia promotes the production of ROS by affecting the first-phase of glucose-induced insulin secretion through the suppression of GAPDH activity<sup>[33]</sup>. However, the outcomes of this study should be further evaluated with a larger sample size as the current study was conducted with a very limited number of diabetic patients. Although the overall pattern of ROS secretion in other chronic metabolic diseases is similar to the current study, a stringent sample selection with a

statistically required sample size will add value to future studies. Moreover, this study was conducted with a conventional yet highly specific and sensitive technique which is laborious in nature due to two critical processes, namely the neutrophil isolation and measurement of ROS. Opting for a much-advanced technique such as flow cytometer using unfractionated peripheral blood will potentially cut down the tedious laboratory procedures and allow investigation of a larger sample size. Nonetheless, harnessing the semi-activated neutrophils in DM could serve as an auxiliary therapy that maintains the oxidative/anti-oxidative balance and integrity of the immune system.

Our pilot study employed a sensitive and specific method, a chemiluminescence technique to measure the ROS production by neutrophils from non-diabetic individuals and diabetic patients. Neutrophils from diabetic patients showed a constitutive and elevated level of respiratory burst compared to non-diabetic individuals in resting and activated conditions. The data from this preliminary study revealed an inherent disability of diabetic-derived neutrophils in regulating ROS secretion; however, such a pathological condition should be verified by a larger sample size and well-designed research study.

## ACKNOWLEDGMENTS

This study was conducted by postgraduate students enrolled in the immunobiology postgraduate course (SMK5301), session 2011/2012 as part of the practical requirement. We thank all patients and healthy individuals whom voluntarily participated in this preliminary study.

## COMMENTS

### Background

Diabetes mellitus is a metabolic disorder that is often associated with vital organ failure if untreated. The excessive production and release of reactive oxygen species (ROS) are known as key factors that contribute to diabetic complications. Particularly, neutrophils are much more susceptible to the hyperglycemic condition and significantly contribute to the severity of diabetic complications by spontaneously releasing an abundant amount of ROS.

### Research frontiers

Controlling the excessive release of ROS by neutrophils could serve as a promising tool in managing or preventing diabetic complications. Inhibiting a relevant signaling pathway that governs the release or production of excessive ROS can be exploited therapeutically.

### Innovations and breakthroughs

The current preliminary study strengthens the existing laboratory and clinical data where the vulnerability of diabetic patients' derived neutrophils to release an excessive amount of ROS at both resting and activated conditions was noted.

### Applications

Reduction of ROS release in neutrophils could serve as an auxiliary therapy in managing diabetic complications.



## Terminology

ROS: Reactive oxygen species.

## Peer-review

This research article is very well written, clearly presenting augmented ROS production in type 2 diabetes mellitus patients' neutrophils for the first time.

## REFERENCES

- 1 **Di Carlo E**, Forni G, Lollini P, Colombo MP, Modesti A, Musiani P. The intriguing role of polymorphonuclear neutrophils in antitumor reactions. *Blood* 2001; **97**: 339-345 [PMID: 11154206 DOI: 10.1182/blood.V97.2.339]
- 2 **Ramasamy R**, Krishna K, Maqbool M, Vellasamy S, Sarmadi VH, Abdullah M, Vidyadaran S. The effect of human mesenchymal stem cell on neutrophil oxidative burst. *Malays J Med Health Sci* 2010; **6**: 11-17
- 3 **Ramasamy R**, Maqbool M, Mohamed AL, Noah RM. Elevated neutrophil respiratory burst activity in essential hypertensive patients. *Cell Immunol* 2010; **263**: 230-234 [PMID: 20471005 DOI: 10.1016/j.cellimm.2010.04.004]
- 4 **Wolff SP**, Jiang ZY, Hunt JV. Protein glycation and oxidative stress in diabetes mellitus and ageing. *Free Radic Biol Med* 1991; **10**: 339-352 [PMID: 1855674 DOI: 10.1016/0891-5849(91)90040-A]
- 5 **Wolff SP**, Dean RT. Glucose autooxidation and protein modification. The potential role of 'autooxidative glycosylation' in diabetes. *Biochem J* 1987; **245**: 243-250 [PMID: 3117042]
- 6 **Kaneto H**, Kajimoto Y, Miyagawa J, Matsuoka T, Fujitani Y, Umayahara Y, Hanafusa T, Matsuzawa Y, Yamasaki Y, Hori M. Beneficial effects of antioxidants in diabetes: possible protection of pancreatic beta-cells against glucose toxicity. *Diabetes* 1999; **48**: 2398-2406 [PMID: 10580429 DOI: 10.2337/diabetes.48.12.2398]
- 7 **Godin DV**, Wohaieb SA, Garnett ME, Goumeniouk AD. Antioxidant enzyme alterations in experimental and clinical diabetes. *Mol Cell Biochem* 1988; **84**: 223-231 [PMID: 3231224 DOI: 10.1007/BF00421057]
- 8 **Davies MG**, Hagen PO. The vascular endothelium. A new horizon. *Ann Surg* 1993; **218**: 593-609 [PMID: 8239773 DOI: 10.1097/0000658-199321850-00003]
- 9 **Fukuto JM**, Hobbs AJ, Ignarro LJ. Conversion of nitroxyl (HNO) to nitric oxide (NO) in biological systems: the role of physiological oxidants and relevance to the biological activity of HNO. *Biochem Biophys Res Commun* 1993; **196**: 707-713 [PMID: 8240347 DOI: 10.1006/bbrc.1993.2307]
- 10 **Beckman JS**, Crow JP. Pathological implications of nitric oxide, superoxide and peroxynitrite formation. *Biochem Soc Trans* 1993; **21**: 330-334 [PMID: 8395426 DOI: 10.1042/bst0210330]
- 11 **Kaur H**, Halliwell B. Evidence for nitric oxide-mediated oxidative damage in chronic inflammation. Nitrotyrosine in serum and synovial fluid from rheumatoid patients. *FEBS Lett* 1994; **350**: 9-12 [PMID: 8062931 DOI: 10.1016/0014-5793(94)00722-5]
- 12 **Biswas SK**, Bhelwa AP, Upadhyay AU, George A, Nath N. Status of nitric oxide free radicals in diabetic neutrophils: effect of diabetic serum factor on the generation of these species in normal neutrophils and their relation to lysosomal degranulation. *Indian J Biochem Biophys* 1993; **30**: 293-296 [PMID: 8144175]
- 13 **Evans JL**, Goldfine ID, Maddux BA, Grodsky GM. Are oxidative stress-activated signaling pathways mediators of insulin resistance and beta-cell dysfunction? *Diabetes* 2003; **52**: 1-8 [PMID: 12502486 DOI: 10.2337/diabetes.52.1.1]
- 14 **Hand WL**, Hand DL, Vasquez Y. Increased polymorphonuclear leukocyte respiratory burst function in type 2 diabetes. *Diabetes Res Clin Pract* 2007; **76**: 44-50 [PMID: 16959366 DOI: 10.1016/j.diabres.2006.07.015]
- 15 **Omori K**, Ohira T, Uchida Y, Ayilavarapu S, Batista EL, Yagi M, Iwata T, Liu H, Hasturk H, Kantarci A, Van Dyke TE. Priming of neutrophil oxidative burst in diabetes requires preassembly of the NADPH oxidase. *J Leukoc Biol* 2008; **84**: 292-301 [PMID: 18390927 DOI: 10.1189/jlb.1207832]
- 16 **Daoud AK**, Tayyar MA, Fouda IM, Harfeil NA. Effects of diabetes mellitus vs. in vitro hyperglycemia on select immune cell functions. *J Immunotoxicol* 2009; **6**: 36-41 [PMID: 19519161 DOI: 10.1080/15476910802604564]
- 17 **Wong RK**, Pettit AI, Davies JE, Ng LL. Augmentation of the neutrophil respiratory burst through the action of advanced glycation end products: a potential contributor to vascular oxidant stress. *Diabetes* 2002; **51**: 2846-2853 [PMID: 12196480 DOI: 10.2337/diabetes.51.9.2846]
- 18 **Stefano GB**, Challenger S, Kream RM. Hyperglycemia-associated alterations in cellular signaling and dysregulated mitochondrial bioenergetics in human metabolic disorders. *Eur J Nutr* 2016; Epub ahead of print [PMID: 27084094 DOI: 10.1007/s00394-016-1212-2]
- 19 **Coughlan MT**, Thorburn DR, Penfold SA, Laskowski A, Harcourt BE, Sourris KC, Tan AL, Fukami K, Thallas-Bonke V, Nawroth PP, Brownlee M, Bierhaus A, Cooper ME, Forbes JM. RAGE-induced cytosolic ROS promote mitochondrial superoxide generation in diabetes. *J Am Soc Nephrol* 2009; **20**: 742-752 [PMID: 19158353 DOI: 10.1681/ASN.2008050514]
- 20 **Maqbool M**, Vidyadaran S, George E, Ramasamy R. Human mesenchymal stem cells protect neutrophils from serum-deprived cell death. *Cell Biol Int* 2011; **35**: 1247-1251 [PMID: 21649586 DOI: 10.1042/CBI20110070]
- 21 **Santegoets SJ**, van den Eertwegh AJ, van de Loosdrecht AA, Scheper RJ, de Gruijl TD. Human dendritic cell line models for DC differentiation and clinical DC vaccination studies. *J Leukoc Biol* 2008; **84**: 1364-1373 [PMID: 18664532 DOI: 10.1189/jlb.0208092]
- 22 **Babior BM**. Oxygen-dependent microbial killing by phagocytes (first of two parts). *N Engl J Med* 1978; **298**: 659-668 [PMID: 24176 DOI: 10.1056/NEJM19780323981205]
- 23 **Lehmeyer JE**, Snyderman R, Johnston RB. Stimulation of neutrophil oxidative metabolism by chemotactic peptides: influence of calcium ion concentration and cytochalasin B and comparison with stimulation by phorbol myristate acetate. *Blood* 1979; **54**: 35-45 [PMID: 444673]
- 24 **Sklar LA**, Hyslop PA, Oades ZG, Omann GM, Jesaitis AJ, Painter RG, Cochrane CG. Signal transduction and ligand-receptor dynamics in the human neutrophil. Transient responses and occupancy-response relations at the formyl peptide receptor. *J Biol Chem* 1985; **260**: 11461-11467 [PMID: 2995337]
- 25 **Webster RO**, Hong SR, Johnston RB, Henson PM. Biological effects of the human complement fragments C5a and C5ades Arg on neutrophil function. *Immunopharmacology* 1980; **2**: 201-219 [PMID: 6254906 DOI: 10.1016/0162-3109(80)90050-8]
- 26 **Repine JE**, White JG, Clawson CC, Holmes BM. The influence of phorbol myristate acetate on oxygen consumption by polymorphonuclear leukocytes. *J Lab Clin Med* 1974; **83**: 911-920 [PMID: 4421315]
- 27 **Dewald B**, Baggiolini M. Platelet-activating factor as a stimulus of exocytosis in human neutrophils. *Biochim Biophys Acta* 1986; **888**: 42-48 [PMID: 3017443 DOI: 10.1016/0167-4889(86)90069-8]
- 28 **Kaibuchi K**, Takai Y, Nishizuka Y. Cooperative roles of various membrane phospholipids in the activation of calcium-activated, phospholipid-dependent protein kinase. *J Biol Chem* 1981; **256**: 7146-7149 [PMID: 7251589]
- 29 **Okamura N**, Uchida M, Ohtsuka T, Kawanishi M, Ishibashi S. Diverse involvements of Ni protein in superoxide anion production in polymorphonuclear leukocytes depending on the type of membrane stimulants. *Biochem Biophys Res Commun* 1985; **130**: 939-944 [PMID: 2992509 DOI: 10.1016/0006-291X(85)91705-X]
- 30 **Houstis N**, Rosen ED, Lander ES. Reactive oxygen species have a causal role in multiple forms of insulin resistance. *Nature* 2006; **440**: 944-948 [PMID: 16612386 DOI: 10.1038/nature04634]
- 31 **Alba-Loureiro TC**, Munhoz CD, Martins JO, Cerchiaro GA, Scavone C, Curi R, Sannomiya P. Neutrophil function and metabolism in individuals with diabetes mellitus. *Braz J Med Biol Res* 2007; **40**: 1037-1044 [PMID: 17665039 DOI: 10.1590/S0100-879X2006005000143]
- 32 **Costa D**, Marques AP, Reis RL, Lima JL, Fernandes E. Inhibition



of human neutrophil oxidative burst by pyrazolone derivatives.  
*Free Radic Biol Med* 2006; **40**: 632-640 [PMID: 16458194 DOI:  
10.1016/j.freeradbiomed.2005.09.017]

- 33 **Ceriello A**, Motz E. Is oxidative stress the pathogenic mechanism

underlying insulin resistance, diabetes, and cardiovascular disease?  
The common soil hypothesis revisited. *Arterioscler Thromb Vasc  
Biol* 2004; **24**: 816-823 [PMID: 14976002 DOI: 10.1161/01.  
ATV.0000122852.22604.78]

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*World Journal of Diabetes*

Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China  
Telephone: +86-10-85381891

Fax: +86-10-85381893

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## Gestational diabetes mellitus: Screening with fasting plasma glucose

Mukesh M Agarwal

Mukesh M Agarwal, Department of Pathology, College of Medicine, UAE University, PO Box 17666, Al Ain, United Arab Emirates

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**Correspondence to:** Mukesh M Agarwal, MD, FCAP, Professor, Department of Pathology, College of Medicine, UAE University, Khalifa Bin Zayed Street, Tawam Hospital Campus, PO Box 17666, Al Ain, United Arab Emirates. [magarwal7@gmail.com](mailto:magarwal7@gmail.com)  
 Telephone: +971-3-7672000  
 Fax: +971-3-7671966

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### Abstract

Fasting plasma glucose (FPG) as a screening test for gestational diabetes mellitus (GDM) has had a checkered history. During the last three decades, a few

initial anecdotal reports have given way to the recent well-conducted studies. This review: (1) traces the history; (2) weighs the advantages and disadvantages; (3) addresses the significance in early pregnancy; (4) underscores the benefits after delivery; and (5) emphasizes the cost savings of using the FPG in the screening of GDM. It also highlights the utility of fasting capillary glucose and stresses the value of the FPG in circumventing the cumbersome oral glucose tolerance test. An understanding of all the caveats is crucial to be able to use the FPG for investigating glucose intolerance in pregnancy. Thus, all health professionals can use the patient-friendly FPG to simplify the onerous algorithms available for the screening and diagnosis of GDM - thereby helping each and every pregnant woman.

**Key words:** Gestational diabetes mellitus; Screening; Diagnosis; Fasting capillary glucose; Fasting plasma glucose

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**Core tip:** The algorithms for the screening and diagnosis of gestational diabetes mellitus (GDM), advocated by various expert panels, are demanding for both the caregiver and the care-receiver: The widely accepted approach of screening all pregnant women with the oral glucose tolerance test is time-consuming, expensive and unfeasible in most countries. Over three decades of research, summarized in this review, suggests that the fasting plasma glucose can simplify the approach to GDM - only if all the limitations of using it are clearly understood.

Agarwal MM. Gestational diabetes mellitus: Screening with fasting plasma glucose. *World J Diabetes* 2016; 7(14): 279-289 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v7/i14/279.htm> DOI: <http://dx.doi.org/10.4239/wjd.v7.i14.279>



## INTRODUCTION

For many years, gestational diabetes mellitus (GDM) was defined as hyperglycemia first discovered during pregnancy. However, due to the recent epidemic of type 2 diabetes mellitus afflicting numerous younger women in the child-bearing age, this traditional definition has been redefined. The World Health Organization (WHO)<sup>[1]</sup> classifies hyperglycemia first identified in pregnancy as: (1) diabetes mellitus in pregnancy; and (2) GDM. GDM generally refers to milder hyperglycemia and lesser degree of glucose intolerance occurring in the latter half of pregnancy, which usually does not persist after delivery in most patients. According to the American Diabetes Association (ADA), GDM is diabetes diagnosed in the second or third trimester of pregnancy that is not type 1 or type 2 diabetes mellitus (T1DM or T2DM)<sup>[2]</sup>. T1DM is caused by absolute insulin deficiency with positive autoimmune markers which destroy pancreatic  $\beta$ -cells, while T2DM is caused by insulin resistance or relative insulin deficiency. Clearly, GDM is distinct from both these types of diabetes<sup>[2]</sup>. The reason to segregate women with DM who become pregnant is because these women have more severe complications compared to pregnant women with GDM. However, GDM is also associated with many maternal (preeclampsia, increase in cesarean sections, birth injuries) and fetal problems (macrosomia, hypoglycemia, shoulder dystocia)<sup>[3]</sup>. After delivery, patients with GDM are at a risk of developing T2DM in the mother and childhood obesity in the neonate<sup>[4]</sup>. The pathogenesis of GDM is well-understood. The hormonal changes of pregnancy cause insulin resistance; most mothers compensate by increasing insulin secretion—something women with GDM are not able to do.

The diagnosis of GDM is confirmed by the 75 g or 100 g oral glucose tolerance test (OGTT). Screening for GDM can be done by: (1) clinical risk factors; (2) the glucose challenge test (GCT); or (3) the OGTT. Even though the ideal screening method is without consensus, screening generally involves a one-step or a two-step approach. In the one step approach, all patients undergo the diagnostic OGTT. In the two-step algorithm, screening is done either by: (1) assessing the clinical risk factors; or (2) the glucose GCT usually at 24-28 wk gestation, when venous plasma glucose is measured one hour after 50 g oral glucose. Patients who have clinical risk factors or exceed a specific GCT screening threshold undergo the diagnostic OGTT. However, due to an array of recommendations available (Table 1), the screening and diagnosis of GDM remains without consensus. Often, the obstetric and endocrine associations within the same country support markedly dissimilar protocols for GDM leading to major inconsistencies in the approach to GDM globally<sup>[5]</sup>. In 2010, the International Association of Diabetes and Pregnancy Study Groups (IADPSG) proposed a unified approach for screening and diagnosis of GDM advocating the 2 h, 75 g OGTT for all pregnant women at 24-28 wk<sup>[6]</sup>. Since its suggested glucose OGTT thresholds were based on the elaborate Hyperglycemia

and Adverse Pregnancy Outcome (HAPO) study<sup>[7]</sup>, the IADPSG approach has been accepted by many reputed expert panels [e.g., WHO, ADA and the Australasian Diabetes in Pregnancy Society (ADIPS)], but not all the major health organizations around the world [e.g., the American College of Obstetricians and Gynecologists (ACOG) and the National Institute for Health and Care Excellence (NICE)].

Due to their wide acceptance, the IADPSG is best suited to be accepted world-wide. The International Federation of Gynecology and Obstetrics which has accepted the IADPSG criteria has issued a pragmatic guide for four categories: High, upper-middle, low-middle and low resource countries. Thus, depending on the resources, the IADPSG recommendations can be universally applied with modifications<sup>[8]</sup>.

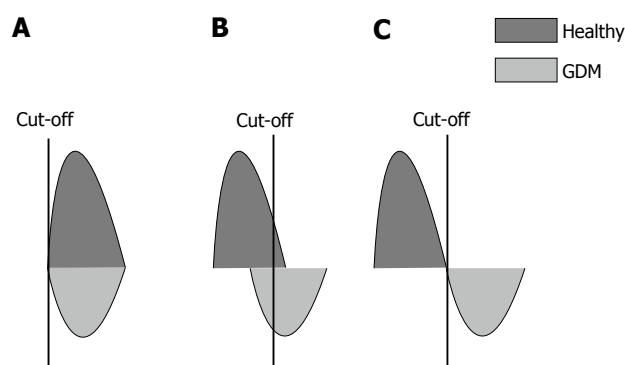
## FEATURES OF A GOOD SCREENING TEST

The conventional thinking is that screening tests should be very sensitive (*i.e.*, without false negatives) so that no patient with the disease is missed, while diagnostic tests should be specific (*i.e.*, without false positives) so the diagnosis can be confirmed in all patients with potential disease (initially picked up by the sensitive screening test). In any population, a perfect screening test would separate all the patients with disease (defined by clinical criteria or a "gold-standard" test, *e.g.*, bone marrow stainable iron for iron deficiency anemia and OGTT for GDM) from all the healthy subjects. Thus, for GDM, the positive screening test should identify most women with GDM (true positives; the number of women picked up from all women with GDM will depend on the sensitivity) along with some women without GDM (false positives; the number of women falsely identified with GDM from amongst women without GDM will depend on the specificity), and the specific diagnostic test (OGTT in this case) will separate the true and false positives. However, usually due to overlap of the screening test results among the diseased and healthy population, choosing an appropriate cut-off (depending on the sensitivity/specificity combination desired) for the screening test would help it to become highly sensitive with minimum loss of specificity - something that may not be possible if there is a major overlap between the diseased and healthy populations. Thus, a screening test with poor specificity, *i.e.*, too many healthy testing as diseased (being over the threshold for diagnosis due to too many false positives) would have to proceed with the test needed to confirm the diagnosis. This would make the screening test of little use since its main function is to avoid the cumbersome and expensive diagnostic test. A screening test with 0% specificity, *i.e.*, when there is a total overlap of the diseased and healthy populations (Figure 1A), is useless. When there is less overlap between diseased and non-diseased, the performance of the screening test will be better (Figure 1B). The ideal state, when there is total segregation between diseased and healthy

**Table 1** Diagnostic criteria for gestational diabetes mellitus (by country)

Organization	Use prevalent in	Year	Glucose load (g)	F mmol/L	1-h mmol/L	2-h mmol/L	3-h mmol/L	Values for diagnosis
NDDG <sup>1</sup>	United States/North America	1979	100	5.8	10.6	9.2	8.1	≥ 2
ADA (C and C)	United States/North America	2003 (1982)	100	5.3	10.0	8.6	7.8	≥ 2
ADA <sup>2</sup>	United States/North America	2011	75	5.1	10.0	8.5	--	≥ 1
CDA	Canada	2013	75	5.3	10.6	9.0	--	≥ 1
EASD	Europe	1991	75	6.0	--	9.0	--	≥ 1
NICE	United Kingdom	2015	75	5.6	--	7.8	--	≥ 1
ADIPS <sup>2</sup>	Australasia	2014	75	5.1	10.0	8.5	--	≥ 1
NZSSD	New Zealand	1998	75	5.5	--	9.0	--	≥ 1
JDS <sup>2</sup>	Japan	2013	75	5.1	10.0	8.5	--	≥ 1
IADPSG	Multiple countries	2010	75	5.1	10.0	8.5	--	≥ 1
WHO <sup>2</sup>	Multiple countries	2013	75	5.1	10.0	8.5	--	≥ 1

<sup>1</sup>Endorsed by American College of Obstetricians and Gynecologists; <sup>2</sup>Same as IADPSG. ADA: American Diabetes Association; ADIPS: Australasian Diabetes in Pregnancy Society; CDA: Canadian Diabetes Association; C and C: Carpenter-Coustas; EASD: European Association for the Study of Diabetes; JDS: Japan Diabetes Society; NDDG: National Diabetes Data Group; NICE: National Institute for Health and Care Excellence; NZSSD: New Zealand Society for the study of diabetes; WHO: World Health Organization; IADPSG: International Association of Diabetes and Pregnancy Study Groups.



**Figure 1** Effect healthy and diseased populations overlap on screening test performance (A-C). GDM: Gestational diabetes mellitus.

populations (Figure 1C), is a situation which is almost never achieved.

## IS SCREENING FOR GDM WARRANTED?

In the past, there were extensive and acrimonious debates about the screening for GDM. Many questions have been raised: Should we screen pregnant women for GDM at all? Should screening be based on clinical risk factors only? Is screening with GCT a valid and potentially the best approach to screening? What is the most cost-effective way to screen for GDM? How good are other screening methods like fasting plasma glucose (FPG), glycated albumin and HBA1c?

Screening for GDM does not meet many of the screening criteria set by the United Kingdom National Screening Committee, which is a modified version of the WHO criteria for assessing proposed screening programs<sup>[9]</sup>. Despite this, most preeminent professional societies, e.g., ADA and WHO, recommend screening for GDM. In 2002, a thorough review by the Health Technology Program, United Kingdom<sup>[10]</sup> concluded, "On balance, the present evidence suggests that we should not have universal

screening, but a highly selective policy, based on age and overweight (of patients)".

In 2008, after reviewing all the evidence, the preeminent United States Preventive Services Task Force (USPSTF) determined that the evidence was insufficient to assess the benefits and harms of screening for GDM. However, in 2014, the USPSTF (after another comprehensive follow-up review) advised that asymptomatic women after 24 wk of gestation should be screened for GDM, though before 24 wk, the evidence was insufficient<sup>[11]</sup>.

If it is decided to screen for GDM, there is debate about the best way to screen for GDM. Though originally screening via risk-factors (age, obesity, family history of DM, GDM in previous pregnancy, non-white race, previous miscarriage/stillbirth/fetal malformation/preeclampsia/macrosomia) was widely recommended, many studies recommend otherwise: A recent comprehensive study found that this approach would miss a third of women with GDM<sup>[12]</sup>. In another recent commentary about the ideal way to screen, an editorial in a preeminent journal argued that whichever way one looks at it, there is no justification for either risk-factor or GCT based screening<sup>[13]</sup>. Their advice: The OGTT should be used for both screening and diagnosis of GDM-as recommended by the IADPSG. Though the cost of screening increases and more women are labelled as GDM, the St. Carlos study confirms that in the long term it is cheaper due to the fewer complications<sup>[14]</sup>.

Many laboratory screening tests have been tried for screening of GDM. They are direct glucose measurements (FPG, GCT) or indirect measurements of glucose (HBA1c, fructosamine). Newer markers (insulin, irisin, galanin, adiponectin, sex hormone-binding globulin, C-reactive protein, fibrinectin, glycosylated fibrinactin, ferritin, glycated CD59) especially in early pregnancy have been tried to predict GDM later in pregnancy. However, only GCT and FPG have shown some promise. The holy grail of screening for GDM has yet to be found.

## OGTT AS A GOLD STANDARD FOR DIAGNOSIS OF GDM AND DIAGNOSTIC CRITERIA

As pointed earlier, all the expert panels agree that the OGTT is the "gold standard" for GDM diagnosis. The OGTT has many drawbacks, the most serious flaw being that it is not reproducible<sup>[15]</sup>. It is expensive, time consuming and quite demanding for both the patient and the laboratory; furthermore, it is also not physiologic, quite unpleasant, uncorrected for body weight and its predictive value changes with ethnicity due to varying prevalence of GDM<sup>[16]</sup>. As a diagnostic test for DM in non-pregnant adults, many arguments have been made for keeping the OGTT<sup>[17]</sup> or avoiding it<sup>[15]</sup>. Due to the numerous problems of the OGTT, since 1997, the ADA favors the FPG with a lower threshold (7.0 mmol/L), rather than the OGTT for the diagnosis of DM in non-pregnant adults-even though this approach has its critics<sup>[18]</sup>. However, there has been no debate about the OGTT as a diagnostic test for GDM. Despite the potential of nausea and vomiting in pregnant women<sup>[19]</sup>, the OGTT remains the cornerstone for diagnosis of GDM. Though many alternatives for screening of GDM have been explored, however, only the OGTT is currently acceptable as the diagnostic test.

Additional tests with OGTT may help to improve its performance. Measuring insulin with the 100 g OGTT may identify a subgroup of women who do not meet the ACOG criteria for GDM as they have only one abnormal glucose value. It has been found that women who have raised one hour serum glucose post oral glucose may need more intensive treatment<sup>[20]</sup>. The diagnosis of GDM using OGTT in pregnancy are further compounded by the variation in guidelines of the various preeminent expert panels for the glucose load used (75 g vs 100 g) and, as mentioned earlier, in varying diagnostic glucose thresholds suggested for diagnosis. Thus, a pregnant woman has the potential for undergoing the onerous OGTT three times: First one at booking, second one at between 24-28 wk, and the third one post-partum, six weeks after delivery.

## FPG AS A SCREENING TEST

Over time, the definition of GDM, laboratory methods for glucose, and the screening and diagnostic criteria of GDM have evolved. Initially, in 1985, an anecdotal report<sup>[21]</sup> first used fasting blood glucose (along with glycosuria) for screening pregnant women. The interest in FPG surged when the expert committee of the ADA preferred using the FPG with lower thresholds rather than the OGTT for DM diagnosis in non-pregnant adults. In 1999, once the WHO approved this ADA approach, FPG became even more accepted and popular. Later, some studies while studying GDM screening, accidentally found that the FPG may have value<sup>[22]</sup>. The first comprehensive study on FPG as a screening test was conducted by Sacks *et al*<sup>[23]</sup>.

In fact, Professor David Sacks due to his extensive initial and subsequent pioneering and iconic studies should be credited for putting FPG as a screening test for GDM on the world map.

## FPG AS A SCREENING TEST: ADVANTAGES AND DISADVANTAGES

As a screening test for GDM, the FPG is very appealing: It cheap, reliable, reproducible, does not produce vomiting as seen with the OGTT/GCT. Thus, it can be administered in women unable to tolerate glucose drink and it takes less time than GCT. Using the FPG make GDM screening and diagnosis patient friendly<sup>[24]</sup>. However, the value of FPG for GDM screening remains uncertain. It is also not without problems. Incomplete fasting or an inability to fast for at least 8 h may not be easy for some pregnant women. In many poorer countries, multiple studies confirm that women find it hard to come to a clinic fasting. In some countries, fasting becomes hard if not impossible due to cultural beliefs that pregnant women should not fast for a long time, and commuting to the clinic takes an inordinately long time making it hard to fast. Often, the dropout rate is high when a pregnant woman is asked to come again for an OGTT after the clinic appointment. In some Asian populations, the FPG is inherently much lower (than Caucasians) but the postprandial is very high<sup>[25]</sup>. Thus, in India the authority on GDM, Diabetes in Pregnancy Study Group India advocates "a single-step procedure", *i.e.*, the 2-h glucose without fasting glucose for the screening and diagnosis of GDM<sup>[26]</sup>.

## THE PROBLEMS OF STUDIES EVALUATING THE FPG IN SCREENING

The potential problems in interpreting studies of screening with FPG are as follows: (1) numerous studies evaluating FPG screening have a pre-selection bias. Patients are selected on the basis of clinical history or positive GCT; then, they undergo an OGTT which is not done on all patients and compared to the FPG. This creates a higher prevalence of GDM, improving the predictive value of the FPG<sup>[27]</sup>. The entire population must undergo both the screening and diagnostic test. Any surrogate screening test should not be assessed using a biased population and applying findings to a healthy population<sup>[28]</sup>; (2) results in different populations have varying prevalence and cannot be compared. However, standardized procedures and ethnicity customization will improve reproducibility; (3) FPG performance is also difficult to compare between studies as differing criteria are used for the diagnosis of GDM; (4) studies should use FPG independent of the OGTT to evaluate its performance. Using the FPG of the OGTT is erroneous as it assumes FPG is reproducible, which may not be so; and (5) in most reports, FPG performance is compared to the OGTT rather than examining how the test predicts

**Table 2** Studies about fasting plasma glucose as a screening test

<i>n</i>	Cut-off mmol/L	Se (%)	Sp (%)	GDM (%)	AUC	Glucose load (g)	OG criteria <sup>1</sup>	Ref.
Without selection								
bias								
5010	4.5	81.5	54	7.6	--	75	WHO-1985	Reichelt <i>et al</i> <sup>[37]</sup>
558	4.8	81	76	10.2	0.897	100	ADA	Perucchini <i>et al</i> <sup>[27]</sup>
942	4.1	> 70		13	0.766	75	WHO-1985	Tam <i>et al</i> <sup>[38]</sup>
1685	4.7	78.1	32.2	19.8	0.639	75	WHO-1999	Agarwal <i>et al</i> <sup>[39]</sup>
500	4.7	88	95	7.2	--	100	C and C-1982	Poomalar <i>et al</i> <sup>[41]</sup>
In early pregnancy								
4507	4.6	80	43	6.7	0.7	75	Sacks	Sacks <i>et al</i> <sup>[46]</sup>
708	4.7	79.9	27.5	25.9	0.579	75	WHO-1999	Agarwal <i>et al</i> <sup>[47]</sup>
4876	4.4	79	46.9	2.8	0.72	100	C and C 100-g OGTT	Riskin-Mashiah <i>et al</i> <sup>[49]</sup>
17186	4.3	84	29	12.4	--	75	IADPSG	Zhu <i>et al</i> <sup>[51]</sup>
486	--	47.2	77.4	10.9	0.623	--	FPG > 5.1 mmol/L	Yeral <i>et al</i> <sup>[53]</sup>

<sup>1</sup>Fasting plasma glucose (FPG) threshold with Specificity (Sp) corresponding to Sensitivity (Se) about 80%. AUC: Area under receiver operating characteristic curve; C and C: Carpenter-Coustan; ADA: American Diabetes Association; WHO: World Health Organization; IADPSG: International Association of Diabetes and Pregnancy Study Groups; OGTT: Oral glucose tolerance test; GDM: Gestational diabetes mellitus.

poor health outcome. It has been suggested that the “gold-standard” for screening tests would be a universally agreed-on set of pregnancy outcomes<sup>[29]</sup>.

## BIASED REPORTS ON GDM SCREENING WITH FPG

As pointed earlier, to evaluate any screening test, the screening test and the diagnostic test must be done in the entire cohort. Otherwise, the evaluation is not accurate. In FPG screening, this is often not the case since only the positive screen patients (by clinical risk-factors or the 50-g GCT) undergo the OGTT. The FPG performance is compared to these fewer preselected patients who undergo the OGTT. These earlier studies by Sacks *et al*<sup>[23]</sup>, de Aguiar *et al*<sup>[30]</sup>, Agarwal *et al*<sup>[31,32]</sup>, Rey *et al*<sup>[33]</sup>, Soheilykhah *et al*<sup>[34]</sup>, Senanayake *et al*<sup>[35]</sup> and Juutinen *et al*<sup>[36]</sup> suffer from this drawback (Table 2).

## UNBIASED STUDIES ON FPG SCREENING FOR GDM

In 1998, the first comprehensive unbiased study of FPG screening for GDM was from Brazil (Table 2). Based on this study, Brazil became one of the few countries that recommend using FPG as a screening test for GDM in their national guidelines. Reichelt *et al*<sup>[37]</sup> analyzed the value of FPG as a screening test for GDM in 5010 women. The FPG performed well in the 16 (0.3%) women with frank diabetes (2-h > 11.1 mmol/L). However, in most of the other 363 (7.2%) women with reduced and compromised glucose tolerance (GIGT, 2-h = 7.8-11.0 mmol/L), despite the author's claim, the performance was less than satisfactory. At their ideal cut-off of 4.7 mmol/L, both the sensitivity and specificity for women with GIGT were too low to be of any use (68.0%). If the threshold was decreased to 4.5 mmol/L, the sensitivity and specificity would be 81.5% and 54%; 51% women were less than this threshold. Thus, approximately one in

two of their pregnant patients would have to proceed to the diagnostic OGTT to pick up 8 of 10 women with GDM. The increased number of false positives would make FPG as an inefficient screening test for GDM.

The 1999 study by Perucchini *et al*<sup>[27]</sup> evoked a lot of interest in FPG since it was published in the preeminent British Medical Journal. In 520 women who were pregnant the FPG performed better than the OGCT (Carpenter and Coustan criteria, C and C, using the 3-h, 100 g OGTT). FPG as a screening test had a good overall sensitivity and specificity. However, the number of women was small and the cohort was very small.

In 2000, Tam *et al*<sup>[38]</sup> from Hong Kong, inspired by the Reichelt study, compared 50 g glucose challenge, FPG, random glucose and fructosamine in 942 women who were pregnant. The prevalence of GDM (1980 WHO criteria) was 13%; since the area under a receiver operating characteristic curve (AUC) for GCT, FPG and 2-h glucose were similar, due to its simplicity, they recommended universal screening using FPG (cut-off 4.1 mmol/L) rather than the GCT.

In 2005, in one study involving 1685 pregnant women (WHO GDM criteria for the 75 g OGTT)<sup>[39]</sup>, we found that the elevated number of women testing as false-positive made the FPG an inefficient test for GDM screening. Subsequently, a year later, we showed that<sup>[40]</sup> the variation in FPG performance may be due to the differing diagnostic criteria used for the diagnosis of GDM. The performance of FPG as a screening test with 4 different diagnostic criteria (using the same 75 g OGTT) was compared. In 4602 women, the FPG efficiency as a screening test was a function of the criteria used for diagnosis; it was excellent when the ADA-2003 criteria were used for diagnosis. With the other three criteria (WHO, ADIPS, European Association of Study on diabetes), at a satisfactory 85% sensitivity, the increased FPR and low specificity limited the value of FPG in screening.

More recently in 2013, Poomalar *et al*<sup>[41]</sup> compared FPG and GCT as screening tests. They found (like Perucchini's study) that the ROC curve for FPG was better



than GCT. However, their numbers were also small (500 women) and one is uncertain about the randomization of their subjects and the number of women missed during the study period.

## FPG AS A SCREENING TEST FOR GDM IN EARLY PREGNANCY

In screening for GDM in early pregnancy (Table 2), two questions arise: (1) can the diagnosis of GDM be made in early pregnancy? and (2) how to interpret a raised FPG in early pregnancy.

In 2014, as stated earlier, the USPSTF concluded that the evidence was not enough to assess the balance of benefits and harms of screening for GDM in asymptomatic pregnant women before 24 wk of pregnancy<sup>[11]</sup>. It has been shown that higher first trimester FPG levels increase the risk of adverse pregnancy outcomes<sup>[42]</sup>. Some experts have cogently argued that the IADPSG recommendation (endorsed by WHO) that an FPG of 5.1-6.9 mmol/L be classified as GDM in pregnancy cannot be accepted as there are no controlled trials that address the benefits of diagnosing and treating GDM in early pregnancy<sup>[43]</sup>. Even the ADA does not support the IADPSG view to diagnose GDM in early pregnancy.

Physiologically, in non-obese women, there is a fall in FPG in early pregnancy (median 0.11 mmol/L between 6-10 wk gestation), thereafter the glucose levels decrease little. Eight of ten studies showed a decrease in the first trimester<sup>[44]</sup>. A more recent study observed the same finding about FPG, while in the second to the third trimester, most studies have shown that the FPG changed little<sup>[45]</sup>. Thus, the thresholds used in third trimester for GDM diagnosis-on a theoretical basis-cannot be used in the first trimester.

The controversies about GDM diagnosis in early pregnancy notwithstanding, different studies have addressed this issue about FPG screening in early pregnancy with mixed results. Sacks *et al*<sup>[46]</sup> concluded that in their 5557 women during the first prenatal visit, despite good compliance, the poor specificity of FPG made it an inefficient test for screening for GDM. Similar conclusions were drawn by us<sup>[47]</sup> in a highrisk population. However, Corrado *et al*<sup>[48]</sup> observed that a FPG  $\geq 5.1$  mmol/L predicts GDM in later pregnancy. Similarly, Riskin-Mashiah *et al*<sup>[49]</sup> found that FPG may be used as a screening test to assess risk, but not as a diagnostic test in early pregnancy: A higher FPG in the first trimester, even though in the normal range, constituted a risk for GDM in later pregnancy. Alunni *et al*<sup>[50]</sup> found that implementing FPG (and HbA1c) screening in early pregnancy, nearly doubled the incidence of GDM and predicted the need for more pharmacotherapy. An extensive study by Zhu *et al*<sup>[51]</sup>, involving 17186 women from China, showed that the first prenatal visit FPG correlated strongly with GDM at 24-28 wk gestation; however, they also assert that FPG  $\geq 5.1$  mmol/L should not be used to make a diagnosis of GDM in early pregnancy. They found that

besides gestational age, chronological age also affects the FPG level as an independent variable. A study in 2004, on 246 women, found that FPG does not predict GDM in later pregnancy<sup>[52]</sup>.

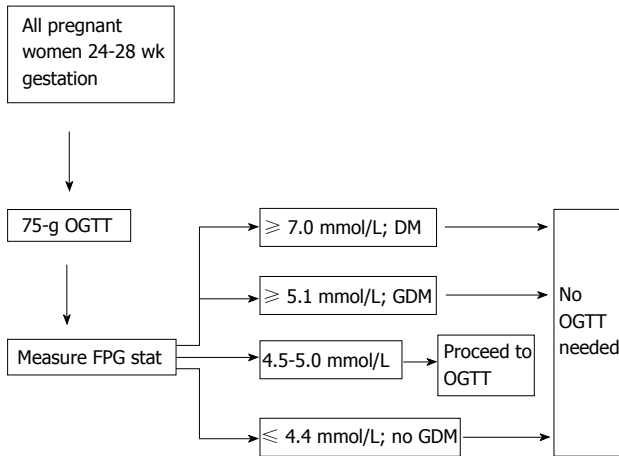
In 2014, Yeral *et al*<sup>[53]</sup> measured the FPG of 736 women during early pregnancy (1<sup>st</sup> visit) and randomized them (at 2<sup>nd</sup> visit) into: (1) the two-step 50 g GCT followed by 3-h, 100 g OGTT for positive results; and (2) the one step 2-h, 75 g OGTT repeating the tests in late pregnancy for women testing negative. GDM was diagnosed by Carpenter and Coustan criteria for 100 g OGTT and IADPSG criteria for the 75 g OGTT (in both second visit and late pregnancy). Within each cohort, the sensitivity in early pregnancy of 50 g GCT and 75 g OGTT was 68.2% and 87.1%, respectively. However, they reported the consolidated performance of FPG in early pregnancy (sensitivity 47.2%) for GDM diagnosed by the different criteria in the two groups. Since FPG performance is a function of the diagnostic criteria<sup>[40]</sup>, individual performance of FPG is needed in each group to interpret their results further. Furthermore, the FPG results cannot be compared to other studies as two OGTT gold-standards were used.

In summary, most studies agree that the FPG in early pregnancy can predict risk for GDM in late pregnancy and possibly the need for medical therapy (and insulin). However, its poor specificity makes it an inappropriate test for screening test in early pregnancy - if and once the experts agree that GDM can be diagnosed in early pregnancy at all.

## FASTING CAPILLARY GLUCOSE AS A SCREENING TEST FOR GDM

Few studies have addressed the value of fasting capillary glucose (FCG) as a screening test for GDM. There is an excellent correlation between fasting capillary glucose and fasting venous fasting glucose in pregnant women<sup>[54]</sup>; thus, the fasting capillary glucose shares the same performance characteristics as the fasting venous glucose.

Three studies have reported on the value of FCG as a screening test for GDM. These studies were done in populations of countries at low risk (Sweden)<sup>[55]</sup>, moderate risk (Canada)<sup>[56]</sup> and highrisk (United Arab Emirates)<sup>[57]</sup> for GDM. Both studies in the high risk population and lowrisk population showed a similar AUC (87%), sensitivity (86%), specificity (55%) at FCG thresholds of 4.0 mmol/L and 4.7 mmol/L, respectively. The study from Canada was designed differently; it used FCG in preselected patients who tested positive with 50 g GCT with the specific aim to define a threshold which could rule of GDM without the need for an OGTT. The AUC was modest at 0.67. The fasting capillary glucose was positively associated with OGTT glucose values, and inversely associated with insulin sensitivity and pancreatic beta cell function. However, due to the overlap of FCG in the GDM and non-GDM populations, it could not be used to rule out GDM reliably. All three studies show that, like



**Figure 2 Suggested algorithm for gestational diabetes mellitus screening.** OGTT: Oral glucose tolerance test; FPG: Fasting plasma glucose; GDM: Gestational diabetes mellitus; DM: Diabetes mellitus.

the FPG, the poor specificity precludes using FCG for GDM screening<sup>[58]</sup> since too many healthy women testing positive (false positives) would need the diagnostic OGTT. However, using the FCG (like FPG) may be of value to avoid a number of OGTTs needed, as discussed in the next section.

## USING THE FPG TO DECREASE THE NUMBER OF OGTTs

The two-threshold method: Screening every pregnant woman for GDM with the OGTT, as advised by all expert panels (WHO, ADA, ACOG, CDA), is very demanding for the patient, the laboratory and the health-delivery system. Hence, there is a need for simpler, alternative screening tests. Screening tests are sensitive or specific, and generally, as the sensitivity increases, the specificity decreases and vice versa. So, to get the best of a screening test's performance, Henderson, a chemical pathologist, advocates using two-thresholds<sup>[59]</sup>. In short, two threshold values, instead of one cut-off (as is the common practice), are utilized for the screening (e.g., fasting glucose for GDM). The higher cut-off, the specificity of which is innately increased, is used to "rule-in" the disease (GDM here); while the lower cut-off with its inherently increased sensitivity is used to "rule-out" the disease. Subjects with results in between these two selected cut-offs, are "indefinite" and would need the diagnostic test. All subjects above the higher threshold and below the lower threshold, do not need to be evaluated further. Thus, the FPG can be used to limit the number of OGTTs in any population. The author of this review is a chemical pathologist; thus, being aware of the literature in clinical chemistry has been applying this method to GDM.

Thus, since 2000, in our UAE population, we have used the two threshold "rule-in and rule-out" algorithm GDM in multiple studies<sup>[32,39,40,60,61]</sup> (Table 3). Depending on the FPG result, the OGTT can be avoided completely: (1) the upper chosen FPG cut-off, "rules-in" GDM with

100% specificity, and (2) the lesser FPG cut-off selected "rules-out" GDM with variable sensitivity. Table 3 shows that between 25%-70% women would not need the OGTT using this algorithm. Studies from China<sup>[62]</sup> and Brazil<sup>[63]</sup> have shown similar results.

Therefore, using this approach, the FPG could tentatively avoid 33.0%-50.0% OGTTs, depending on the GDM diagnostic criteria<sup>[40,61]</sup>. Most countries still using the GCT for screening and OGTT for diagnosis may find it more cost-effective and simpler to switch to the OGTT for screening and diagnosis using the FPG decreasing the number of OGTTs needed-only after making sure this FPG-OGTT algorithm works in their population.

Rationale of using the 5.1 mmol/L and 4.4 mmol/L FPG thresholds: As per the criteria of the IADPSG, an FPG  $\geq 5.1$  mmol/L (independent of the other two values of the 75 g OGTT) confirms GDM. The lower cut-off of 4.4 mmol/L is derived from the elaborate HAPO study<sup>[7]</sup> which found that pregnancy outcomes were good when the FPG was  $\leq 4.4$  mmol/L. This approach is shown diagrammatically in Figure 2.

Shortcomings of the 2 threshold approach: There is a major difference in fasting glucose levels between 15 different centers distributed globally as shown by the HAPO study<sup>[64]</sup>. In some Asian populations, the FPG is very low but the postprandial is very high<sup>[25]</sup>. Thus, the suggested approach may, in many populations, may not circumvent too many OGTTs.

Another concern with approach is laboratory turnaround time (time taken to get the FPG result). If too long, this algorithm cannot be used. To decrease the turnaround time, a glucometer has been used to measure the fasting capillary glucose. The glucometer FCG has been found to be as good as the laboratory FPG with the excellent diagnostic correlation ( $\kappa = 0.95$ ) for GDM<sup>[57]</sup>.

## COST OF SCREENING WITH FPG

Few studies analyzing cost of FPG screening compared to other screening methods are available. One study compared eight screening strategies<sup>[65]</sup>. It found that when the risk of GDM in a population was between 1.0%-4.2%, FPG followed by the OGTT was the cost-effective method. When risk was less (or more), other strategies were better. They also comment on another very important aspect of screening: Acceptance rates. The percentage of women who would undergo the screening test was as follows: OGTT, 40%; FPG, 50%; GCT, 70%; and RPG, 90%.

Another study<sup>[66]</sup>, calculated the costs of three strategies: The 2-step (GCT + 100 g OGTT), the 1-step (75 g OGTT) and FPG of the OGTT to limit the number of OGTTs. Of the three strategies, the last one was the ideal approach.

## FPG AS A POST-PARTUM SCREENING TEST AFTER DELIVERY

Since GDM is a marker for diabetes mellitus after delivery,

**Table 3** Studies using fasting glucose to avoid oral glucose tolerance test

OGTTs circumvented (n, %)	Thresholds (lower and higher) mmol/L	OGTT (g)	Diagnostic criteria	Comments	Ref.
50.9	4.4 and 5.3	100	ADA (C and C)	Biased sampling: Preselected by clinical/GCT	Agarwal <i>et al</i> <sup>[32]</sup>
30.1	4.7 and --	75	WHO-1999	Only lower threshold used to rule out GDM	Agarwal <i>et al</i> <sup>[39]</sup>
63.8	4.9 and 7.0	75	ADA (C and C)	FPG screening dependent on GDM criteria	Agarwal <i>et al</i> <sup>[40]</sup>
68.5	4.9 and 7.0	75	ADA (C and C)	Glucometer used for FPG	Agarwal <i>et al</i> <sup>[60]</sup>
50.1	4.7 and 7.0	75	ADA (C and C)	Fasting capillary glucose used	Agarwal <i>et al</i> <sup>[57]</sup>
50.6	4.4 and 5.1	75	IADPSG	Pooled data from 4 studies	Agarwal <i>et al</i> <sup>[61]</sup>
50.3	4.4 and 5.1	75	IADPSG	Data from China corroborating UAE data	Zhu <i>et al</i> <sup>[62]</sup>
61.0	4.4 and 5.1	75	IADPSG	Data from Brazil corroborating UAE data	Trujillo <i>et al</i> <sup>[63]</sup>
57.0	4.4 and 5.1	75	IADPSG	Thresholds applied to HAPO Study	Agarwal <i>et al</i> <sup>[77]</sup>

OGTT: Oral glucose tolerance test; ADA: American Diabetes Association; WHO: World Health Organization; IADPSG: International Association of Diabetes and Pregnancy Study Groups; C and C: Carpenter-Coustan; GCT: Glucose challenge test; GDM: Gestational diabetes mellitus; FPG: Fasting plasma glucose; UAE: United Arab Emirates; HAPO: Hyperglycemia and Adverse Pregnancy Outcome.

it is obligatory to find the state of glucose tolerance in the immediate postnatal period and after long-term follow-up. All major guidelines recommend testing the mother 6-12 wk after birth of the baby; however there is a variation in the recommendations of the tests to use: FPG or the OGTT. The ADA and CDA recommend the OGTT, the NICE advocates FPG while the WHO and ACOG maintain that either test is acceptable<sup>[67]</sup>. The OGTT is more sensitive and picks up a higher number of women with dysglycemia, but the compliance is less (between 30%-70%). A ten year study showed that fasting glucose missed up 10% of women with DM and 60% women with impaired glucose tolerance<sup>[68]</sup>. We have reported that both tests show similar estimates for DM but widely discordant rates for glucose intolerance depending on the criteria used for DM diagnosis<sup>[69]</sup>. Kim *et al*<sup>[67]</sup> cogently argue that the decision could be based on the criteria used to pick up GDM antepartum. Thus, if the less stringent criteria are used (e.g., IADPSG) which picks up more women with dysglycemia post-partum, it may be better to use the FPG since the disparity between the two will decrease when women with lesser degrees of glucose intolerance are identified antepartum<sup>[69]</sup>.

## OTHER STUDIES ABOUT FPG AND GDM

Atilano *et al*<sup>[22]</sup> found that an abnormal FPG  $\geq 5.8$  mmol/L predicted GDM much better than an abnormal GCT. In this study, very high FPG values showed an excellent positive predictive value (96%), but the corresponding sensitivity at these high levels would remain poor. However, their patient population was pre-selected by an abnormal GCT giving a high prevalence of GDM of 22% and there are many doubts if the conclusions can be universally applied. Herrera *et al*<sup>[70]</sup> in 324 patients with GDM (75 g OGTT at 24-28 wk by C and C criteria) found 7.0% women who had isolated elevated FPG were more likely to need hypoglycemic agents, have higher body mass index and be Black or Hispanic. Another study compared FPG and hs-CRP in the first trimester and found the former was more sensitive and the latter more specific<sup>[71]</sup>. A higher maternal fasting glucose during 4-12

gestational weeks in 57454 women was associated with an increased birth weight and birth length of the offspring during 6-12 mo of the infant's life<sup>[72]</sup>.

## STUDIES CHALLENGING THE USE OF FPG FOR GDM SCREENING

Many studies have found the FPG to be an inadequate test for GDM. Most of these studies are from South Asia, where women have lower FPG than their Caucasian counterparts. Balaji *et al*<sup>[73]</sup> found that FPG was inadequate as a screening test in their 1643 subjects from South India when compared to the WHO-1999 criteria. A threshold of 5.1 mmol/L had a sensitivity of just 24.0%. In another study<sup>[36]</sup> on 435 Finnish women with GDM (by the older criteria of the fourth International Conference-Workshop on GDM, a FPG threshold of 4.8 mmol/L picked up just 69.6% of the women with GDM). However, despite the poor sensitivity, FPG predicted the need for insulin. A 2003 study from Japan<sup>[74]</sup>, found that in 749 Japanese women, a FPG threshold of 85.0 mg/dL had a sensitivity of 71.4% and 75.0 in first and second trimester, respectively; however, there were just 22 (2.9%) women with GDM (Japan Diabetes Association criteria).

## FPG AND THE ROLE OF THE LABORATORY

### FPG as a screening test for GDM: Other reviews

All reviews analyzing FPG as a screening test comment about the problem of analyzing the results. There is a lot of inconsistency and wide variation in the sensitivity and specificity found by these studies because of the ethnicity of the population, local prevalence and the diagnostic criteria used. In November 2012, the Agency for Healthcare Research and Quality of the United States Department of Health and Human Services<sup>[3]</sup>, Maryland analyzed 7 studies on FPG to screen for GDM. They were unable to make any definite conclusions about the FPG as a screening test. They found that the FPG was not good at predicting an abnormal OGTT. In

2010, Virally *et al*<sup>[75]</sup> looked at 8 reports commenting on screening for GDM using FPG. Their conclusion was that due to the heterogeneity the studies were impossible to compare; some were in highrisk populations and the diagnostic criteria were very variable. They were critical of the fact that none of the GDM studies related to perinatal outcomes. In 2013, the USPSTF published a systemic review of screening tests for GDM<sup>[29]</sup>. At a FPG threshold of 4.7 mmol/L, the sensitivity was similar to GCT. However, the positive likelihood ratio (LR) of 1.8 compared unfavorably to the positive LR of 5.9 of the GCT. Thus, they concluded that FPG and GCT were good at identifying women who do not have GDM but the FPG was not as good as GCT to identify women who have GDM. They also found that FPG did not diagnose GDM as frequently in Asian as non-Asian women.

## CONCLUSION

In general, for the screening of GDM, the FPG is more sensitive than specific, *i.e.*, it is better at "ruling-out" than "ruling-in" GDM<sup>[76]</sup>. Its performance is highly dependent upon the ethnicity of the population, the GDM prevalence, the diagnostic criteria and the FPG thresholds used. If these screening thresholds are kept low, the FPG will identify most women with GDM, but also an excessive number of women without GDM (due to poor specificity). Therefore, at an acceptable sensitivity, the poor specificity and high-false positive rate limit its usefulness as a screening test. However, as shown by studies originally from UAE, and reproduced by studies from China and similar studies from Brazil, it can still be very useful to decide if the OGTT is needed for diagnosis. Then, the FPG can help to reduce the number of onerous OGTTs required by nearly half<sup>[61,77]</sup>; however, 5%-15% patients with GDM would be missed, potentially women with lesser degrees of glucose intolerance - so health care will not be compromised. In summary, once its caveats are clearly understood, the FPG can simplify the screening and diagnosis of GDM. Thus, by circumventing the OGTT, the FPG can relieve many pregnant woman in the demanding work-up of glucose intolerance.

## REFERENCES

- 1 **World Health Organization.** Diagnostic Criteria and Classification of Hyperglycaemia First Detected in Pregnancy. Geneva: WHO Press, 2013
- 2 **American Diabetes Association.** Classification and Diagnosis of Diabetes. *Diabetes Care* 2016; **39** Suppl 1: S13-S22 [PMID: 26696675]
- 3 **Hartling L,** Dryden DM, Guthrie A, Muise M, Vandermeer B, Aktary WM, Pasichnyk D, Seida JC, Donovan L. Screening and diagnosing gestational diabetes mellitus. *Evid Rep Technol Assess* (Full Rep) 2012; **(210)**: 1-327 [PMID: 24423035 DOI: 10.1016/S0140-6736(09)60731-5]
- 4 **Bellamy L,** Casas JP, Hingorani AD, Williams D. Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. *Lancet* 2009; **373**: 1773-1779 [PMID: 19465232]
- 5 **Agarwal MM,** Dhatt GS, Othman Y. Gestational diabetes: differences between the current international diagnostic criteria and implications of switching to IADPSG. *J Diabetes Complications* 2015; **29**: 544-549 [PMID: 25837380 DOI: 10.1016/j.jdiacomp.2015.03.006]
- 6 **Metzger BE,** Gabbe SG, Persson B, Buchanan TA, Catalano PA, Damm P, Dyer AR, Leiva Ad, Hod M, Kitzmiller JL, Lowe LP, McIntyre HD, Oats JJ, Omori Y, Schmidt MI. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. *Diabetes Care* 2010; **33**: 676-682 [PMID: 20190296 DOI: 10.2337/dc09-1848]
- 7 **Metzger BE,** Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, Coustan DR, Hadden DR, McCance DR, Hod M, McIntyre HD, Oats JJ, Persson B, Rogers MS, Sacks DA. Hyperglycemia and adverse pregnancy outcomes. *N Engl J Med* 2008; **358**: 1991-2002 [PMID: 18463375 DOI: 10.1056/NEJMoa070943]
- 8 **Hod M,** Kapur A, Sacks DA, Hadar E, Agarwal M, Di Renzo GC, Cabero Roura L, McIntyre HD, Morris JL, Divakar H. The International Federation of Gynecology and Obstetrics (FIGO) Initiative on gestational diabetes mellitus: A pragmatic guide for diagnosis, management, and care. *Int J Gynaecol Obstet* 2015; **131** Suppl 3: S173-S211 [PMID: 26433807 DOI: 10.1016/S0020-7292(15)30007-2]
- 9 **UK National Screening Committee.** Criteria for appraising the viability, effectiveness and appropriateness of a screening programme. 2015. Available from: URL: <https://www.gov.uk/government/publications/evidence-review-criteria-national-screening-programmes>
- 10 **Scott DA,** Loveman E, McIntyre L, Waugh N. Screening for gestational diabetes: a systematic review and economic evaluation. *Health Technol Assess* 2002; **6**: 1-161 [PMID: 12433317 DOI: 10.3310/hta6110]
- 11 **Moyer VA.** Screening for gestational diabetes mellitus: U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med* 2014; **160**: 414-420 [PMID: 24424622 DOI: 10.7326/M13-2905]
- 12 **Cosson E,** Benbara A, Pharisien I, Nguyen MT, Revaux A, Lormeau B, Sandre-Banon D, Assad N, Pillegand C, Valensi P, Carbillion L. Diagnostic and prognostic performances over 9 years of a selective screening strategy for gestational diabetes mellitus in a cohort of 18,775 subjects. *Diabetes Care* 2013; **36**: 598-603 [PMID: 23150287 DOI: 10.2337/dc12-1428]
- 13 **Simmons D,** Moses RG. Gestational diabetes mellitus: to screen or not to screen?: Is this really still a question? *Diabetes Care* 2013; **36**: 2877-2878 [PMID: 24065839 DOI: 10.2337/dc13-0833]
- 14 **Duran A,** Sáenz S, Torrejón MJ, Bordiú E, Del Valle L, Galindo M, Perez N, Herraiz MA, Izquierdo N, Rubio MA, Runkle I, Pérez-Ferre N, Cusiñuallpa I, Jiménez S, García de la Torre N, Fernández MD, Montañez C, Familiar C, Calle-Pascual AL. Introduction of IADPSG criteria for the screening and diagnosis of gestational diabetes mellitus results in improved pregnancy outcomes at a lower cost in a large cohort of pregnant women: the St. Carlos Gestational Diabetes Study. *Diabetes Care* 2014; **37**: 2442-2450 [PMID: 24947793 DOI: 10.2337/dc14-0179]
- 15 **Davidson MB.** Counterpoint: the oral glucose tolerance test is superfluous. *Diabetes Care* 2002; **25**: 1883-1885 [PMID: 12351497 DOI: 10.2337/diacare.25.10.1883]
- 16 **Hanna FW,** Peters JR. Screening for gestational diabetes; past, present and future. *Diabet Med* 2002; **19**: 351-358 [PMID: 12027921 DOI: 10.1046/j.1464-5491.2002.00684.x]
- 17 **Tuomilehto J.** Point: a glucose tolerance test is important for clinical practice. *Diabetes Care* 2002; **25**: 1880-1882 [PMID: 12351496 DOI: 10.2337/diacare.25.10.1880]
- 18 **Dekker JM,** Balkau B. Counterpoint: impaired fasting glucose: The case against the new American Diabetes Association guidelines. *Diabetes Care* 2006; **29**: 1173-1175 [PMID: 16644660 DOI: 10.2337/dc05-2220]
- 19 **Agarwal MM,** Punnoose J, Dhatt GS. Gestational diabetes: problems associated with the oral glucose tolerance test. *Diabetes Res Clin Pract* 2004; **63**: 73-74 [PMID: 14693415 DOI: 10.1016/j.diabres.2003.08.005]
- 20 **Di Cianni G,** Seghieri G, Lencioni C, Cuccuru I, Anichini R, De



- Bellis A, Ghio A, Tesi F, Volpe L, Del Prato S. Normal glucose tolerance and gestational diabetes mellitus: what is in between? *Diabetes Care* 2007; **30**: 1783-1788 [PMID: 17416793 DOI: 10.2337/dc07-0119]
- 21 **Mortensen HB**, Mølsted-Pedersen L, Kühl C, Backer P. A screening procedure for diabetes in pregnancy. *Diabetes Metab* 1985; **11**: 249-253 [PMID: 4043492]
- 22 **Atilano LC**, Lee-Parritz A, Lieberman E, Cohen AP, Barbieri RL. Alternative methods of diagnosing gestational diabetes mellitus. *Am J Obstet Gynecol* 1999; **181**: 1158-1161 [PMID: 10561637 DOI: 10.1016/S0002-9378(99)70100-6]
- 23 **Sacks DA**, Greenspoon JS, Fotheringham N. Could the fasting plasma glucose assay be used to screen for gestational diabetes? *J Reprod Med* 1992; **37**: 907-909 [PMID: 1460607]
- 24 **Rey E**. Screening for gestational diabetes mellitus. A simple test may make it easier to study whether screening is worthwhile. *BMJ* 1999; **319**: 798-799 [PMID: 10496805 DOI: 10.1136/bmj.319.7213.798]
- 25 **Wijeyaratne CN**, Ginige S, Arasalingam A, Egodage C, Wijewardhena K. Screening for gestational diabetes mellitus: the Sri Lankan experience. *Ceylon Med J* 2006; **51**: 53-58 [PMID: 17180809]
- 26 **Anjalakshi C**, Balaji V, Balaji MS, Ashalata S, Suganthi S, Arthi T, Thamizharasi M, Seshiah V. A single test procedure to diagnose gestational diabetes mellitus. *Acta Diabetol* 2009; **46**: 51-54 [PMID: 18830559 DOI: 10.1007/s00592-008-0060-9]
- 27 **Perucchini D**, Fischer U, Spinass GA, Huch R, Huch A, Lehmann R. Using fasting plasma glucose concentrations to screen for gestational diabetes mellitus: prospective population based study. *BMJ* 1999; **319**: 812-815 [PMID: 10496823 DOI: 10.1136/bmj.319.7213.812]
- 28 **Sox HC**, Higgins MC, Owens DK. In: Medical Decision Making. 2nd ed. Wiley-Blackwell. NJ, USA, 2013
- 29 **Donovan L**, Hartling L, Muise M, Guthrie A, Vandermeer B, Dryden DM. Screening tests for gestational diabetes: a systematic review for the U.S. Preventive Services Task Force. *Ann Intern Med* 2013; **159**: 115-122 [PMID: 23712349 DOI: 10.7326/0003-4819-159-2-201307160-00657]
- 30 **de Aguiar LG**, de Matos HJ, Gomes MB. Could fasting plasma glucose be used for screening highrisk outpatients for gestational diabetes mellitus? *Diabetes Care* 2001; **24**: 954-955 [PMID: 11347763 DOI: 10.2337/diacare.24.5.954]
- 31 **Agarwal MM**, Hughes PF, Ezimokhai M. Screening for gestational diabetes in a highrisk population using fasting plasma glucose. *Int J Gynaecol Obstet* 2000; **68**: 147-148 [PMID: 10717820 DOI: 10.1016/S0020-7292(99)00201-5]
- 32 **Agarwal MM**, Hughes PF, Punnoose J, Ezimokhai M. Fasting plasma glucose as a screening test for gestational diabetes in a multi-ethnic, highrisk population. *Diabet Med* 2000; **17**: 720-726 [PMID: 11110505 DOI: 10.1046/j.1464-5491.2000.00371.x]
- 33 **Rey E**, Hudon L, Michon N, Boucher P, Ethier J, Saint-Louis P. Fasting plasma glucose versus glucose challenge test: screening for gestational diabetes and cost effectiveness. *Clin Biochem* 2004; **37**: 780-784 [PMID: 15329316 DOI: 10.1016/j.clinbiochem.2004.05.018]
- 34 **Soheilikhah S**, Rashidi M, Mojibian M, Dara N, Jafari F. An appropriate test for diagnosis of gestational diabetes mellitus. *Gynecol Endocrinol* 2011; **27**: 785-788 [PMID: 21250875 DOI: 10.3109/09513590.2010.540598]
- 35 **Senanayake H**, Seneviratne S, Ariyaratne H, Wijeratne S. Screening for gestational diabetes mellitus in southern Asian women. *J Obstet Gynaecol Res* 2006; **32**: 286-291 [PMID: 16764618 DOI: 10.1111/j.1447-0756.2006.00400.x]
- 36 **Juutinen J**, Hartikainen AL, Bloigu R, Tapanainen JS. A retrospective study on 435 women with gestational diabetes: fasting plasma glucose is not sensitive enough for screening but predicts a need for insulin treatment. *Diabetes Care* 2000; **23**: 1858-1859 [PMID: 11128372 DOI: 10.2337/diacare.23.12.1858]
- 37 **Reichelt AJ**, Spichler ER, Branchtein L, Nucci LB, Franco LJ, Schmidt MI. Fasting plasma glucose is a useful test for the detection of gestational diabetes. Brazilian Study of Gestational Diabetes (EBDG) Working Group. *Diabetes Care* 1998; **21**: 1246-1249 [PMID: 9702428 DOI: 10.2337/diacare.21.8.1246]
- 38 **Tam WH**, Rogers MS, Yip SK, Lau TK, Leung TY. Which screening test is the best for gestational impaired glucose tolerance and gestational diabetes mellitus? *Diabetes Care* 2000; **23**: 1432 [PMID: 10977047 DOI: 10.2337/diacare.23.9.1432b]
- 39 **Agarwal MM**, Dhatt GS, Punnoose J, Koster G. Gestational diabetes in a highrisk population: using the fasting plasma glucose to simplify the diagnostic algorithm. *Eur J Obstet Gynecol Reprod Biol* 2005; **120**: 39-44 [PMID: 15866084 DOI: 10.1016/j.ejogrb.2004.07.034]
- 40 **Agarwal MM**, Dhatt GS, Punnoose J. Gestational diabetes: utility of fasting plasma glucose as a screening test depends on the diagnostic criteria. *Diabet Med* 2006; **23**: 1319-1326 [PMID: 17116182 DOI: 10.1111/j.1464-5491.2006.01987.x]
- 41 **Poomalar GK**, Rangaswamy V. A comparison of fasting plasma glucose and glucose challenge test for screening of gestational diabetes mellitus. *J Obstet Gynaecol* 2013; **33**: 447-450 [PMID: 23815193 DOI: 10.3109/01443615.2013.771156]
- 42 **Riskin-Mashiah S**, Younes G, Damti A, Auslander R. First-trimester fasting hyperglycemia and adverse pregnancy outcomes. *Diabetes Care* 2009; **32**: 1639-1643 [PMID: 19549728 DOI: 10.2337/dc09-0688]
- 43 **McIntyre HD**, Sacks DA, Barbour LA, Feig DS, Catalano PM, Damm P, McElduff A. Issues With the Diagnosis and Classification of Hyperglycemia in Early Pregnancy. *Diabetes Care* 2016; **39**: 53-54 [PMID: 26519336 DOI: 10.2337/dc15-1887]
- 44 **Mills JL**, Jovanovic L, Knopp R, Aarons J, Conley M, Park E, Lee YJ, Holmes L, Simpson JL, Metzger B. Physiological reduction in fasting plasma glucose concentration in the first trimester of normal pregnancy: the diabetes in early pregnancy study. *Metabolism* 1998; **47**: 1140-1144 [PMID: 9751245 DOI: 10.1016/S0026-0495(98)90290-6]
- 45 **Riskin-Mashiah S**, Damti A, Younes G, Auslander R. Normal fasting plasma glucose levels during pregnancy: a hospital-based study. *J Perinat Med* 2011; **39**: 209-211 [PMID: 21241203 DOI: 10.1515/JPM.2010]
- 46 **Sacks DA**, Chen W, Wolde-Tsadik G, Buchanan TA. Fasting plasma glucose test at the first prenatal visit as a screen for gestational diabetes. *Obstet Gynecol* 2003; **101**: 1197-1203 [PMID: 12798525 DOI: 10.1016/S0029-7844(03)00049-8]
- 47 **Agarwal MM**, Dhatt GS, Punnoose J, Zayed R. Gestational diabetes: fasting and postprandial glucose as first prenatal screening tests in a highrisk population. *J Reprod Med* 2007; **52**: 299-305 [PMID: 17506370]
- 48 **Corrado F**, D'Anna R, Cannata ML, Interdonato ML, Pintaudi B, Di Benedetto A. Correspondence between first-trimester fasting glycaemia, and oral glucose tolerance test in gestational diabetes diagnosis. *Diabetes Metab* 2012; **38**: 458-461 [PMID: 22595470 DOI: 10.1016/j.diabet.2012.03.006]
- 49 **Riskin-Mashiah S**, Damti A, Younes G, Auslander R. First trimester fasting hyperglycemia as a predictor for the development of gestational diabetes mellitus. *Eur J Obstet Gynecol Reprod Biol* 2010; **152**: 163-167 [PMID: 20579799 DOI: 10.1016/j.ejogrb.2010.05.036]
- 50 **Alunni ML**, Roeder HA, Moore TR, Ramos GA. First trimester gestational diabetes screening - Change in incidence and pharmacotherapy need. *Diabetes Res Clin Pract* 2015; **109**: 135-140 [PMID: 25958098 DOI: 10.1016/j.diabres.2015.04.027]
- 51 **Zhu WW**, Yang HX, Wei YM, Yan J, Wang ZL, Li XL, Wu HR, Li N, Zhang MH, Liu XH, Zhang H, Wang YH, Niu JM, Gan YJ, Zhong LR, Wang YF, Kapur A. Evaluation of the value of fasting plasma glucose in the first prenatal visit to diagnose gestational diabetes mellitus in china. *Diabetes Care* 2013; **36**: 586-590 [PMID: 23193214 DOI: 10.2337/dc12-1157]
- 52 **Bhattacharya SM**. Fasting or two-hour postprandial plasma glucose levels in early months of pregnancy as screening tools for gestational diabetes mellitus developing in later months of pregnancy. *J Obstet Gynaecol Res* 2004; **30**: 333-336 [PMID: 15238113 DOI: 10.1111/j.1447-0756.2004.00205.x]

- 53 **Yeral MI**, Ozgu-Erdinc AS, Uygur D, Seckin KD, Karsli MF, Danisman AN. Prediction of gestational diabetes mellitus in the first trimester, comparison of fasting plasma glucose, two-step and one-step methods: a prospective randomized controlled trial. *Endocrine* 2014; **46**: 512-518 [PMID: 24282036 DOI: 10.1007/s12020-013-0111-z]
- 54 **Dhatt GS**, Agarwal MM, Othman Y, Nair SC. Performance of the Roche Accu-Chek active glucose meter to screen for gestational diabetes mellitus using fasting capillary blood. *Diabetes Technol Ther* 2011; **13**: 1229-1233 [PMID: 21864017 DOI: 10.1089/dia.2011.0097]
- 55 **Fadl H**, Ostlund I, Nilsson K, Hanson U. Fasting capillary glucose as a screening test for gestational diabetes mellitus. *BJOG* 2006; **113**: 1067-1071 [PMID: 16956338 DOI: 10.1111/j.1471-0528.2006.01032.x]
- 56 **Anderson V**, Ye C, Sermer M, Connelly PW, Hanley AJ, Zinman B, Retnakaran R. Fasting capillary glucose as a screening test for ruling out gestational diabetes mellitus. *J Obstet Gynaecol Can* 2013; **35**: 515-522 [PMID: 23870775 DOI: 10.1016/S1701-2163(15)30909-9]
- 57 **Agarwal MM**, Dhatt GS, Othman Y, Gupta R. Gestational diabetes: fasting capillary glucose as a screening test in a multi-ethnic, highrisk population. *Diabet Med* 2009; **26**: 760-765 [PMID: 19709144 DOI: 10.1111/j.1464-5491.2009.02765.x]
- 58 **Agarwal MM**, Dhatt GS, Bali N. Fasting capillary glucose as a screening test for gestational diabetes mellitus. *BJOG* 2007; **114**: 237-238; author reply 237-238 [PMID: 17305902 DOI: 10.1111/j.1471-0528.2006.01191.x]
- 59 **Henderson AR**. Assessing test accuracy and its clinical consequences: a primer for receiver operating characteristic curve analysis. *Ann Clin Biochem* 1993; **30** (Pt 6): 521-539 [PMID: 8304720 DOI: 10.1177/000456329303000601]
- 60 **Agarwal MM**, Dhatt GS, Safraou MF. Gestational diabetes: using a portable glucometer to simplify the approach to screening. *Gynecol Obstet Invest* 2008; **66**: 178-183 [PMID: 18562798 DOI: 10.1159/000140602]
- 61 **Agarwal MM**, Dhatt GS, Shah SM. Gestational diabetes mellitus: simplifying the international association of diabetes and pregnancy diagnostic algorithm using fasting plasma glucose. *Diabetes Care* 2010; **33**: 2018-2020 [PMID: 20519664 DOI: 10.2337/dc10-0572]
- 62 **Zhu WW**, Fan L, Yang HX, Kong LY, Su SP, Wang ZL, Hu YL, Zhang MH, Sun LZ, Mi Y, Du XP, Zhang H, Wang YH, Huang YP, Zhong LR, Wu HR, Li N, Wang YF, Kapur A. Fasting plasma glucose at 24-28 weeks to screen for gestational diabetes mellitus: new evidence from China. *Diabetes Care* 2013; **36**: 2038-2040 [PMID: 23536582 DOI: 10.2337/dc12-2465]
- 63 **Trujillo J**, Vigo A, Reichelt A, Duncan BB, Schmidt MI. Fasting plasma glucose to avoid a full OGTT in the diagnosis of gestational diabetes. *Diabetes Res Clin Pract* 2014; **105**: 322-326 [PMID: 25037441 DOI: 10.1016/j.diabres.2014.06.001]
- 64 **Sacks DA**, Hadden DR, Maresh M, Deerochanawong C, Dyer AR, Metzger BE, Lowe LP, Coustan DR, Hod M, Oats JJ, Persson B, Trimble ER. Frequency of gestational diabetes mellitus at collaborating centers based on IADPSG consensus panel-recommended criteria: the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study. *Diabetes Care* 2012; **35**: 526-528 [PMID: 22355019 DOI: 10.2337/dc11-1641]
- 65 **Round JA**, Jacklin P, Fraser RB, Hughes RG, Muggleston MA, Holt RI. Screening for gestational diabetes mellitus: cost-utility of different screening strategies based on a woman's individual risk of disease. *Diabetologia* 2011; **54**: 256-263 [PMID: 20809381 DOI: 10.1007/s00125-010-1881-y]
- 66 **Agarwal MM**, Dhatt GS, Othman Y. Gestational diabetes in a tertiary care hospital: implications of applying the IADPSG criteria. *Arch Gynecol Obstet* 2012; **286**: 373-378 [PMID: 22484479 DOI: 10.1007/s00404-012-2324-4]
- 67 **Kim C**, Chames MC, Johnson TR. Identifying post-partum diabetes after gestational diabetes mellitus: the right test. *Lancet Diabetes Endocrinol* 2013; **1**: 84-86 [PMID: 24622307 DOI: 10.1016/S2213-8587(13)70044-5]
- 68 **Myers JE**, Hasan X, Maresh MJ. Post-natal assessment of gestational diabetes: fasting glucose or full glucose tolerance test? *Diabet Med* 2014; **31**: 1133-1137 [PMID: 24841828 DOI: 10.1111/dme.12503]
- 69 **Agarwal MM**, Punnose J, Dhatt GS. Gestational diabetes: implications of variation in post-partum follow-up criteria. *Eur J Obstet Gynecol Reprod Biol* 2004; **113**: 149-153 [PMID: 15063951]
- 70 **Herrera K**, Brustman L, Foroutan J, Scarpelli S, Murphy E, Francis A, Rosenn B. The importance of fasting blood glucose in screening for gestational diabetes. *J Matern Fetal Neonatal Med* 2015; **28**: 825-828 [PMID: 24939625 DOI: 10.3109/14767058.2014.935322]
- 71 **Ozgu-Erdinc AS**, Yilmaz S, Yeral MI, Seckin KD, Erkaya S, Danisman AN. Prediction of gestational diabetes mellitus in the first trimester: comparison of C-reactive protein, fasting plasma glucose, insulin and insulin sensitivity indices. *J Matern Fetal Neonatal Med* 2015; **28**: 1957-1962 [PMID: 25283990 DOI: 10.3109/14767058.2014.973397]
- 72 **Dong L**, Liu E, Guo J, Pan L, Li B, Leng J, Zhang C, Zhang Y, Li N, Hu G. Relationship between maternal fasting glucose levels at 4-12 gestational weeks and offspring growth and development in early infancy. *Diabetes Res Clin Pract* 2013; **102**: 210-217 [PMID: 24257107 DOI: 10.1016/j.diabres.2013.10.017]
- 73 **Balaji V**, Balaji M, Anjalakshi C, Cynthia A, Arthi T, Seshiah V. Inadequacy of fasting plasma glucose to diagnose gestational diabetes mellitus in Asian Indian women. *Diabetes Res Clin Pract* 2011; **94**: e21-e23 [PMID: 21831468 DOI: 10.1016/j.diabres.2011.07.008]
- 74 **Maegawa Y**, Sugiyama T, Kusaka H, Mitao M, Toyoda N. Screening tests for gestational diabetes in Japan in the 1st and 2nd trimester of pregnancy. *Diabetes Res Clin Pract* 2003; **62**: 47-53 [PMID: 14581157]
- 75 **Virally M**, Laloi-Michelin M. Methods for the screening and diagnosis of gestational diabetes mellitus between 24 and 28 weeks of pregnancy. *Diabetes Metab* 2010; **36**: 549-565 [PMID: 21163420 DOI: 10.1016/j.diabet.2010.11.008]
- 76 **Agarwal MM**, Dhatt GS. Fasting plasma glucose as a screening test for gestational diabetes mellitus. *Arch Gynecol Obstet* 2007; **275**: 81-87 [PMID: 16967273]
- 77 **Agarwal MM**, Weigl B, Hod M. Gestational diabetes screening: the low-cost algorithm. *Int J Gynaecol Obstet* 2011; **115** Suppl 1: S30-S33 [PMID: 22099438 DOI: 10.1016/S0020-7292(11)60009-X]

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## Early detection of diabetic kidney disease: Present limitations and future perspectives

Chih-Hung Lin, Yi-Cheng Chang, Lee-Ming Chuang

Chih-Hung Lin, Yi-Cheng Chang, Lee-Ming Chuang, Department of Internal Medicine, National Taiwan University Hospital, Taipei 100, Taiwan

Chih-Hung Lin, Lee-Ming Chuang, Graduate Institute of Clinical Medicine, College of Medicine, National Taiwan University, Taipei 100, Taiwan

Yi-Cheng Chang, Graduate Institute of Medical Genomics and Proteomics, College of Medicine, National Taiwan University, Taipei 100, Taiwan

Yi-Cheng Chang, Institute of Biomedical Science, Academia Sinica, Taipei 115, Taiwan

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**Correspondence to:** Dr. Lee-Ming Chuang, MD, PhD, Department of Internal Medicine, National Taiwan University Hospital, 7, Chung Shan S. Rd, Taipei 100, Taiwan. [leeming@ntu.edu.tw](mailto:leeming@ntu.edu.tw)  
 Telephone: +886-2-23123456  
 Fax: +886-2-23938859

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### Abstract

Diabetic kidney disease (DKD) is one of the most common diabetic complications, as well as the leading cause of chronic kidney disease and end-stage renal disease around the world. To prevent the dreadful consequence, development of new assays for diagnostic of DKD has always been the priority in the research field of diabetic complications. At present, urinary albumin-to-creatinine ratio and estimated glomerular filtration rate (eGFR) are the standard methods for assessing glomerular damage and renal function changes in clinical practice. However, due to diverse tissue involvement in different individuals, the so-called "non-albuminuric renal impairment" is not uncommon, especially in patients with type 2 diabetes. On the other hand, the precision of creatinine-based GFR estimates is limited in hyperfiltration status. These facts make albuminuria and eGFR less reliable indicators for early-stage DKD. In recent years, considerable progress has been made in the understanding of the pathogenesis of DKD, along with the elucidation of its genetic profiles and phenotypic expression of different molecules. With the help of ever-evolving technologies, it has gradually become plausible to apply the thriving information in clinical practice. The strength and weakness of several novel biomarkers, genomic, proteomic and metabolomic signatures in assisting the early diagnosis of DKD will be discussed in this article.

**Key words:** Diabetic kidney disease; Early diagnosis; Genomics; Biomarkers

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**Core tip:** Estimated glomerular filtration rate (eGFR) and albuminuria are currently the standard method for

detecting diabetic kidney disease (DKD). Creatinine-based GFR estimates are affected by muscle mass and diet pattern, as well as the formula chosen. Albuminuria majorly reflects glomerular dysfunction, and is less sensitive to tubulointerstitial and vascular damages. These facts limit the application of eGFR and albuminuria in the early diagnosis of DKD, especially in heterogeneous type 2 diabetic patients. Through the assistance of genetic information for screening of susceptible patients, together with novel biomarkers to reflect diverse renal tissue damage, early diagnosis of DKD could be facilitated.

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## INTRODUCTION

Diabetes mellitus is currently one of the most rapidly-growing "epidemics" around the world. According to the International Diabetes Federation, 415 million people are currently affected by this disease worldwide<sup>[1]</sup>. By the year 2040, the patient number is expected to rise up to 642 million, reaching a global prevalence of 10%<sup>[1]</sup>. This increasing number of patients, mostly with type 2 diabetes mellitus (T2DM), has influenced the rate of diabetic complications, including diabetic kidney disease (DKD). In developed countries, DKD is one of the most common complications of both type 1 diabetes mellitus (T1DM) and T2DM<sup>[2]</sup>, and is also the leading cause of chronic kidney disease (CKD) and end-stage renal disease (ESRD)<sup>[3-5]</sup>. The costs of care for patients with DKD are extremely high, especially after they enter ESRD. In the United States, for the patients covered by Medicare, the average cost per person per year was USD 20000, whereas it was USD 40000 in the younger group (below 65 years of age)<sup>[2]</sup>. This leads to an increasing burden on the finance and health care systems. Therefore, different methods for identification and management of patients with DKD, especially in the early stages, have always been the priority in the research field of diabetic complications. At present, diagnosis of DKD in clinical settings relies upon the assessment of kidney function, usually by calculating estimated glomerular filtration rate (eGFR), and the assessment of kidney damage, usually by checking urinary albumin-to-creatinine ratio [UACR, urine albumin (mg/L)/urine creatinine (mmol/L)] in random spot urine samples<sup>[6]</sup>. Although these tests can be performed easily, they have certain limitations. Therefore, understanding these limitations is important to both clinical applications and the future quest for better diagnostic methods.

## NATURAL HISTORY OF DKD

The first clinical sign suggestive of DKD is glomerular hyperfiltration, which is observed in about 70% and 50% of the patients with T1DM and T2DM, respectively<sup>[7]</sup>. Due to the increased intraglomerular pressure, the elevation in GFR may exceed 120 mL/min per 1.73 m<sup>2</sup><sup>[8]</sup>. In some patients, hyperfiltration is followed by the development of albuminuria. Most patients with T1DM have a normal UACR (< 3.4 mg/mmol) during the first 5 years after the disease onset. In the subsequent 10-15 years, albuminuria develops in some patients, and progresses gradually if no intervention is taken. Once UACR is over 34 mg/mmol, the GFR decreases progressively at a variable rate. Approximately 50% of the patients with UACR > 34 mg/mmol progress to ESRD over a period of 10 years and approximately 75% of the patients over a period of 20 years<sup>[6]</sup>. In patients with T2DM, however, the natural course of DKD is less understood, as the diagnosis is usually delayed by many years. Some patients already display various degrees of albuminuria at the time of diagnosis; however, only 20% of the patients with UACR > 34 mg/mmol progress to ESRD over a period of 20 years<sup>[9,10]</sup>.

## LIMITATIONS OF EGFR

In terms of renal excretory functions, GFR is considered the best overall index. However, due to its time-consuming nature, the measurement of 24-h creatinine clearance to assess GFR is not always easily performed in clinical settings. Instead, to assess renal function, calculating eGFR using serum creatinine level and formulae such as the modification of diet in renal disease [MDRD,  $eGFR = 175 \times \text{standardized Scr}^{-1.154} \times \text{age}^{-0.203} \times 1.212$  (if black)  $\times 0.742$  (if female), where Scr is serum creatinine]<sup>[11]</sup> or the chronic kidney disease epidemiology collaboration [CKD-EPI,  $eGFR = 141 \times \min(\text{Scr}/k, 1)^{\alpha} \times \max(\text{Scr}/k, 1)^{-1.209} \times 0.993^{\text{Age}} \times 1.018$  (if female)  $\times 1.159$  (if black), where k is 0.7 for females and 0.9 for males,  $\alpha$  is -0.329 for females and -0.411 for males, min indicates the minimum of Scr/k or 1, and max indicates the maximum of Scr/k or 1]<sup>[12]</sup> equations has become a routine practice. The National Kidney Foundation uses eGFR to classify stages of CKD<sup>[13]</sup>. Nonetheless, there are some potential flaws in using eGFR as a marker for the early diagnosis of DKD. First, serum creatinine levels are affected by the muscle mass and diet pattern (especially meat intake)<sup>[14,15]</sup>, and therefore may interfere with the eGFR calculation. Second, the formula used may also cause imprecision in certain conditions. The MDRD equations become less reliable in patients with GFR > 60 mL/min per 1.73 m<sup>2</sup><sup>[16,17]</sup>. This would cause a considerable problem in the early diagnosis of DKD, as glomerular hyperfiltration appears early in the course of the disease. The CKD-EPI equation, on the other hand, is more accurate in patients whose GFR is > 90 mL/min per 1.73 m<sup>2</sup><sup>[18]</sup> and is, therefore, preferred when applying



it in patients with diabetes<sup>[6]</sup>. However, Camargo *et al.*<sup>[19]</sup> reported a marked underestimation of GFR calculated with the CKD-EPI equation in diabetic patients compared to healthy individuals. Moreover, the MDRD and CKD-EPI equations have a P30 value between 80% and 90%, which means that the eGFR generated from these equations has, at best, a 90% chance of being within  $\pm 30\%$  of the measured GFR<sup>[2]</sup>. To sum up, caution should be exercised when using eGFR as the sole marker for diagnosis of DKD.

## LIMITATIONS OF ALBUMINURIA

Albuminuria is considered a marker of kidney damage, especially with glomerular dysfunction. An assay for detecting low concentration of urinary albumin was first described in the 1960s<sup>[20]</sup>. When compared with semi-quantitative method, it is more sensitive and specific for disease survey and monitoring. Similar to GFR, measurement of 24-h urine albumin is time-consuming, and adds little to prediction or accuracy<sup>[13,21]</sup>. Therefore, calculating UACR by checking albumin and creatinine levels in random spot urine samples is currently the standard of clinical practice. However, urinary albumin excretion may also increase for reasons other than DKD, such as physical activity, diet pattern, infection, fever, congestive heart failure, marked hyperglycemia, menstruation, and marked hypertension<sup>[22]</sup>. Therefore, the diagnosis of persistent albuminuria is based on abnormal UACR in two out of three specimens collected within a period of 3–6 mo<sup>[6]</sup>.

A crucial point of clinical significance is the discordance between the presence of albuminuria and the decline in renal function. Perkins *et al.*<sup>[23]</sup> reported the development of advanced CKD (GFR < 60 mL/min per 1.73 m<sup>2</sup>) without concomitant progression of albuminuria in patients with T1DM enrolled in the Joslin Kidney Study. In the Third National Health and Nutrition Examination Survey (NHANES III), a normal urinary albumin level was identified in 36% of the 1197 patients with T2DM who had advanced CKD<sup>[24,25]</sup>. In the United Kingdom Prospective Diabetes Study 74, only 49% of the patients with renal impairment had preceding albuminuria<sup>[26]</sup>. In the Developing Education on Microalbuminuria for Awareness of Renal and Cardiovascular Risk in Diabetes study, advanced CKD was noticed in 17% of those with normal UACR<sup>[27]</sup>. This discordance might be caused by the heterogeneous nature of renal injury, especially in T2DM. As mentioned above, albuminuria is a marker of glomerular dysfunction, which is characteristic of DKD in T1DM<sup>[28,29]</sup>. However, glomerulopathy is a less common pathogenesis in DKD of T2DM. In fact, tubulointerstitial and/or vascular lesions are sometimes the major histological changes<sup>[30–32]</sup>. Penno *et al.*<sup>[33]</sup> described a strong association between prevalence of cardiovascular diseases and “non-albuminuric renal impairment”, suggesting a predominance of macroangiopathy as the underlying renal pathology. Further studies are required to clarify this assumption.

## ALTERNATIVE BIOMARKERS

Due to the limitations of eGFR and albuminuria in the early diagnosis of DKD, enormous efforts have been made to investigate and validate alternative biomarkers in recent decades. A tremendous amount of biomarkers have been evaluated for the diagnosis of DKD, and many studies have shown promising preliminary results (Table 1). However, large-scale studies are still required to validate the value of these biomarkers over and above that of eGFR and UACR.

Cystatin C (CysC) is a 13.3 kDa plasma protein freely filtered through the glomerulus. It does not re-enter the bloodstream in an intact form after being re-absorbed and catabolized by tubular cells<sup>[34]</sup>. Validation studies have showed that serum CysC levels are not affected by muscle mass, which is a major defect of creatinine, and are well-correlated with GFR<sup>[35–37]</sup>. In addition, CysC-based GFR estimation is more accurate than creatinine-based estimation when GFR remains > 60 mL/min per 1.73 m<sup>2</sup><sup>[38,39]</sup>, suggesting that CysC might serve as a better marker of glomerular function in the early stages of DKD. However, a greater intra-individual variability compared to serum creatinine<sup>[37]</sup>, together with a higher cost, should be considered before its clinical application.

Neutrophil gelatinase-associated lipocalin (NGAL) is a 25 kDa molecule which belongs to the lipocalin superfamily. It serves as a binder and transporter of small hydrophobic molecules, and a factor of innate antibacterial responses<sup>[40]</sup>. Urinary NGAL is closely related to the severity of renal impairment in various kidney disease. It is considered to play a protective role in such harmful conditions, as it is capable of promoting the proliferation and differentiation of renal cells<sup>[41]</sup>. Yang *et al.*<sup>[42]</sup> reported that urinary NGAL correlated positively with serum CysC and creatinine levels, and inversely with GFR, whereas serum NGAL correlated negatively with serum CysC, in patients with T2DM. Furthermore, urinary NGAL has been shown to correlate positively with the severity of albuminuria in both T1DM<sup>[43]</sup> and T2DM<sup>[42]</sup> patients. In patients with short duration (less than 5 years) of T2DM, Fu *et al.*<sup>[44]</sup> described a positive correlation between urinary NGAL and glomerular hyperfiltration. Such compelling evidences suggest the potential of NGAL as a novel biomarker for the early detection of DKD.

Kidney injury molecule 1 (KIM1) is a transmembrane protein with immunoglobulin-like and mucin domains in its ectodomain. Upregulated expression of KIM1 in renal tubules has been observed in ischemic, toxic, and proteinuric kidney diseases, suggesting its potential role as a marker of renal damage<sup>[45]</sup>. Similar to NGAL, elevated urinary KIM1 concentrations were identified in T2DM patients with glomerular hyperfiltration<sup>[44]</sup>. Nielsen *et al.*<sup>[43]</sup> reported higher urinary KIM1 excretion in patients with T1DM than in healthy controls. Vaidya *et al.*<sup>[46]</sup> showed that lower baseline concentration of urinary KIM1 was predictive of subsequent regression of albuminuria. These results indicate that the role of KIM1 in the early diagnosis of DKD is worth further investigation.

**Table 1** Advantages of novel biomarkers in the early diagnosis of diabetic kidney disease

Biomarker	Validation study design	Sample size	Type of diabetes	Specimen	Advantages	Ref.
CysC	CO	52 <sup>[38]</sup> 30 <sup>[39]</sup>	2	Serum	Not affected by lean body mass Estimates more accurate than creatinine-based ones when GFR > 60 mL/min per 1.73 m <sup>2</sup>	[35-39]
NGAL	CC	112	2	Urine	Indicator of glomerular hyperfiltration	[44]
KIM1	CC	112	2	Urine	Indicator of glomerular hyperfiltration	[44]
NAG	CC	434	1	Urine	Baseline level predicts development of DKD	[51]
	CC	946	2			[52]
8-oxodG	PC	396	2	Urine	Baseline level predicts development of DKD	[59]
Pentosidine	CC	434	1	Urine	Baseline level predicts progression of albuminuria	[51]
TNFR1/2	RC	628	1	Serum	Baseline level predicts development of advanced CKD	[65]
	RC	410	2			[66]

CysC: Cystatin C; NGAL: Neutrophil gelatinase-associated lipocalin; KIM1: Kidney injury molecule 1; NAG: N-acetyl- $\beta$ -(D)-glucosaminidase; 8-oxodG: 8-oxo-7,8-dihydro-2'-deoxyguanosine; TNFR: Tumor necrosis factor receptor; CO: Case-only; CC: Case-control; PC: Prospective cohort; RC: Retrospective cohort; GFR: Glomerular filtration rate; DKD: Diabetic kidney disease; CKD: Chronic kidney disease.

N-acetyl- $\beta$ -(D)-glucosaminidase (NAG) is a 130 kDa lysosomal enzyme located in the brush border of proximal renal tubular cells. Under normal conditions, NAG is excreted in low amounts in urine during the process of exocytosis. Elevated urinary NAG has been observed in various kidney diseases, suggesting a reflection of renal damage<sup>[47,48]</sup>. In patients with diabetes, increased excretion of NAG in urine has been identified to associate with the severity of albuminuria<sup>[49-51]</sup>. Despite inconsistency has been observed in the correlation between urinary NAG and glomerular hyperfiltration<sup>[44]</sup>, results from the studies of Kern *et al.*<sup>[51]</sup> and Hong *et al.*<sup>[52]</sup> have indicated that higher baseline concentrations of urinary NAG were predictive of future development of DKD. On the other hand, lower baseline urinary concentration of urinary NAG was associated with the subsequent regression of albuminuria<sup>[46]</sup>. In addition to DKD, increased excretion of NAG in urine has also been reported to predict macrovascular complications in patients with T2DM<sup>[52-54]</sup>.

Oxidative stress has been considered to play an important part in the pathogenesis of diabetic complications<sup>[55]</sup>. 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) is an oxidized nucleoside - one of the major product of oxidative damage in nuclear and mitochondrial DNA<sup>[56]</sup>. Upon DNA repair, 8-oxodG is directly excreted into urine without further metabolism, so its urine concentration may serve as a generalized index of oxidative stress<sup>[57]</sup>. The study conducted by Hinokio *et al.*<sup>[58]</sup> demonstrated a close correlation between urinary 8-oxodG excretion and the severity of microvascular diabetic complications. In a 5-year cohort study of 532 Japanese patients with T2DM, baseline concentration of urinary 8-oxodG predicted subsequent development of DKD<sup>[59]</sup>, indicating its potential as a predictive marker.

Hyperglycemia irreversibly modifies long-lived macromolecules by forming advanced glycation end products (AGEs), which cause qualitative and quantitative changes of the components of extracellular matrix. By affecting cell adhesion, growth, and matrix accumulation, AGE-induced changes are associated with the pathogenesis of diabetes complications<sup>[60]</sup>. One of the best chemically

characterized AGEs found in human is pentosidine, which has been considered as a marker of formation and accumulation of AGEs<sup>[61]</sup>. Elevated urinary and plasma pentosidine levels were identified in T2DM patients with DKD<sup>[62]</sup>. Both urinary<sup>[51]</sup> and plasma<sup>[63]</sup> pentosidine levels have been demonstrated to correlate positively with the severity of albuminuria in patients with diabetes. In the study conducted by Kern *et al.*<sup>[51]</sup>, baseline urinary pentosidine excretion in patients with T1DM predicted the progression of albuminuria, with a seven-fold increase in risk for every 50% increase in urinary pentosidine.

Tumor necrosis factor (TNF)- $\alpha$  is a key mediator of inflammation and apoptosis. The signal transduction of TNF- $\alpha$  is commenced *via* two distinct receptors, TNF receptor (TNFR) 1 and TNFR2, which are presented in both membrane-bound form and soluble form in serum<sup>[64]</sup>. Serum levels of TNFR1 and TNFR2 were shown to correlate with GFR in patients with diabetes, and was independent of the status of albuminuria<sup>[64]</sup>. Recent studies in both T1DM<sup>[65]</sup> and T2DM<sup>[66]</sup> patients have indicated that plasma TNFR levels were capable of predicting the development of advanced CKD independently over 12 years of follow-up. These evidences suggest that serum concentrations of TNFR1 and TNFR2 may be utilized as predictors of DKD progression.

## GENETIC SUSCEPTIBILITY

Genetic studies provide a powerful tool in the understanding of disease mechanisms. Emerging evidences have suggested that DKD is heritable<sup>[67-69]</sup>. Prior to the deployment of modern high-throughput technologies such as single nucleotide polymorphism microarray analysis and next-generation sequencing, linkage analysis had revealed variants on different chromosomal regions associated with DKD. For instance, variants on chromosome 18q have been identified to be associated with albuminuria and decreased renal function in different ethnic groups<sup>[70,71]</sup>. With the application of genome-wide association studies (GWASs) over the past decade, considerable progress has been made in the

understanding of genetic background of DKD. Genes such as engulfment and cell motility 1<sup>[72-77]</sup>, FERM domain containing 3<sup>[78-81]</sup>, cysteinyl-tRNA synthase<sup>[78,79,81]</sup>, apolipoprotein L3-non-muscle myosin heavy chain 9<sup>[82,83]</sup> have been identified to be associated with the phenotypic presentations of DKD. Other risk loci have also been reported, yet data from different GWASs are not consistent<sup>[84]</sup>. Several fundamental problems remain to be solved before applying these results in clinical practice. First, genetic heterogeneity is always a major consideration when assessing the genetic background of any disease. Replication studies are essential for patients with DKD in different populations. Second, in most GWASs, DKD was defined as the co-existence of hyperglycemia and proteinuria; therefore, it is likely that these results are confounded by patients with renal damage due to causes other than diabetes. Last but not least, the actual functions of many genes which contain loci of risk are still unknown. Further studies are required to elucidate their roles in the pathogenesis of DKD.

## EPIGENETIC MODIFICATIONS

Epigenetic modifications refer to DNA methylation, histone methylation, and histone acetylation, which alter the expression of a gene by changing its accessibility rather than nucleotide sequence<sup>[85]</sup>. In patients with diabetes, multiple factors, such as hyperglycemia, reactive oxygen species, and inflammation, can trigger epigenetic modifications<sup>[86]</sup>. Knowledge about the role of epigenetic modifications in the pathogenesis of DKD is currently very limited; however, since epigenetics is very sensitive to environmental factors, it is plausible that epigenetic imprints are responsible for the "metabolic memory" linked to diabetic complications<sup>[87]</sup>. Hasegawa *et al.*<sup>[88]</sup> demonstrated that differentially methylated genes correlated with fibrogenesis in microdissected tubules obtained from patients with DKD. In a case-control study of 192 Irish patients with T1DM, Bell *et al.*<sup>[89]</sup> reported that methylation at 19 CpG sites in several genes, including *UNC13B*, was associated with the time to development of DKD. Sapienza *et al.*<sup>[90]</sup> identified 187 genes that were differentially methylated on at least two CpG sites among African American and Hispanic diabetic patients with ESRD. Intriguingly, many of these genes have been recognized previously through genome association or transcription profiling studies, and are associated with inflammation, oxidative stress, ubiquitination, fibrosis, drug metabolism, and development of DKD. These results suggest a very close connection between epigenetic modifications and genetic dysregulations in the pathogenesis of DKD.

## MICRORNA PROFILES

MicroRNAs (miRNAs) are small non-coding RNAs composed of 21-25 nucleotides that are produced by genes. By binding to target mRNAs, miRNAs induce degradation of RNAs or, more frequently, repression of protein

translation<sup>[91]</sup>. Being packed within exosomes, miRNAs are stable in serum, plasma, and urine<sup>[92]</sup>. The stability makes miRNAs as potential candidate biomarkers for the non-invasive diagnosis of many diseases<sup>[93]</sup>.

*In vitro* and *in vivo* studies have revealed the potential roles of miRNAs in the pathogenesis of DKD, especially in the early mesangial expansion stage. Changes in the expression of many miRNAs, such as miR-192<sup>[94-97]</sup>, miR-216a<sup>[98]</sup>, miR-377<sup>[99]</sup>, miR-29c<sup>[100]</sup>, miR-200b/c<sup>[101]</sup>, miR-21<sup>[102]</sup>, miR-1207-5p<sup>[103]</sup>, miR-200a<sup>[104]</sup>, and miR-23b<sup>[105]</sup>, have been identified to be involved in the process of extracellular matrix expansion and fibrosis, interaction with transforming growth factor  $\beta$  and other pro-fibrotic genes. Long *et al.*<sup>[106]</sup> identified miR-93 as a novel regulator of vascular endothelial growth factor in *in vitro* and *in vivo* experimental models under hyperglycemic conditions. Fu *et al.*<sup>[107]</sup> described a significant reduction of endogenous miR-25 in rat mesangial cells treated with high glucose concentrations and in the kidneys of diabetic rats associated with increased nicotinamide adenine dinucleotide phosphate hydrogen oxidase (NOX) activity characterized by high NOX4 expression levels. Zhang *et al.*<sup>[108]</sup> reported that over-expression of miR-451, which targets tyrosine3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta and p38 mitogen-activated protein kinase signaling pathways, resulted in reduced glomerular mesangial cell proliferation *in vitro* and *in vivo*. These experimental findings are summarized in Table 2.

The urinary and serum miRNA in patients with DKD have also been profiled. In T1DM patients with albuminuria, Argyropoulos *et al.*<sup>[109]</sup> showed underexpression of urinary miR-323b-5p, miR-221-3p, miR-524-5p, and miR-188-3p, whereas miR-214-3p, miR-92b-5p, hsa-miR-765, hsa-miR-429, miR-373-5p, miR-1913, and miR-638 were overexpressed. On the other hand, an elevation in urinary miR-130a and miR-145 levels, with a reduction in miR-155 and miR-424, were reported by Barutta *et al.*<sup>[110]</sup> in a similar setting. In patients with T2DM, Peng *et al.*<sup>[111]</sup> described a positive correlation between urinary miR-29 levels and the severity of albuminuria.

Expression of miRNAs was also measured in venous blood from Chinese T2DM patients with and without DKD. Using a microarray-based approach, Zhou *et al.*<sup>[112]</sup> confirmed the downregulation of miR-let-7a in the patients with DKD. Intriguingly, the authors also observed that the distribution of a specific variant within *let-7a* (rs1143770) was significantly higher in patients with diabetes than in healthy controls. These results are summarized in Table 3.

## PROTEOMIC SIGNATURES

Proteomics is defined as "the knowledge of the structure, function, and expression of all proteins in the biochemical or biological context of organisms"<sup>[113]</sup>. The most attractive feature of proteomics is that it allows the monitoring of patterns of multiple urine and plasma proteins simultaneously. Considering the sophisticated

**Table 2** *In vitro* and *in vivo* renal cell models demonstrating the potential involvement of miRNAs in development of diabetic kidney disease

miRNA	Species	Specimen	miRNA expression	Mechanism of action	Ref.
miR-192	Mice/Rat	M, Te, KT	Inconsistent results	Interaction with TGFβ-associated and other pro-fibrotic genes	[94-96]
	Human	Te, KT	Reduced		[97]
miR-216a	Mice	M, KT	Elevated		[98]
miR-377	Mice	M, KT	Elevated		[99]
	Human	M			
miR-29c	Mice	P, KT	Elevated		[100]
miR-200b/c	Mice	M, KT	Elevated		[101]
miR-21	Mice	KT	Elevated		[102]
	Human	Te			
miR-1207-5p	Human	P, M, Te	Elevated		[103]
miR-200a	Rat	Te	Reduced		[104]
	Mice	KT			
miR-23b	Mice	KT	Reduced		[105]
	Human	Te, HEK-293A			
miR-93	Mice	P, En, KT	Reduced		[106]
miR-25	Rat	M, KT	Reduced	Regulation of NOX4 expression	[107]
miR-451	Mice	M, KT	Reduced	Targeting YwhaZ and p38 MAPK signaling pathways	[108]

M: Mesangial cells; Te: Tubular epithelial cells; KT: Kidney tissue; P: Podocytes; En: Endothelial cells; TGFβ: Transforming growth factor β; VEGF: Vascular endothelial growth factor; NOX4: Nicotinamide adenine dinucleotide phosphate hydrogen oxidase 4; YwhaZ: Tyrosine3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta; MAPK: Mitogen-activated protein kinase; HEK-293A: Human Embryonic Kidney-293A cells.

nature of DKD, especially in patients with T2DM, it is plausible that early diagnosis of this disease, which relies only on a single biomarker, might eventually fail to reach optimal sensitivity and specificity<sup>[114]</sup>. The role of proteomics in the early diagnosis of DKD, therefore, is worthy of further evaluation.

DN65 is a panel composed of 65 urinary biomarkers, many of which are fragments of type I collagen. In the study conducted by Rossing *et al.*<sup>[115]</sup>, DN65 was capable of distinguishing between diabetic patients without albuminuria from those with DKD. It was also proved to be sensitive and specific in distinguishing DKD from CKD of other etiologies, as well as predicting the progression toward overt DKD in patients with diabetes who had albuminuria over 3 years. First described by Good *et al.*<sup>[116]</sup> in 2010, CKD273 is another panel of 273 urinary peptides and proteins capable of identifying CKD of any cause with excellent sensitivity and specificity. In a cohort of 35 patients with diabetes, Züribig *et al.*<sup>[117]</sup> showed that the CKD273 classifier was capable of detecting those who were at risk of DKD progression up to 5 years prior to development of overt albuminuria. In the Prevention of Renal and Vascular End-stage Disease (PREVEND) cohort, Roscioni *et al.*<sup>[118]</sup> showed that the baseline CKD273 classifier score was independently associated with the progression of albuminuria. In urine samples obtained from 165 patients with T2DM at 9 different centers, Siwy *et al.*<sup>[119]</sup> demonstrated that the classifier could identify DKD patients with high consistency.

## METABOLOMIC SIGNATURES

Metabolomics refers to the identification of low molecular weight intermediate and end-products of cellular functions in a biological sample with nuclear magnetic resonance and mass spectrometry-based profiling

techniques<sup>[120,121]</sup>. As metabolome represents the complete collection of metabolites in an organism, understanding the perturbations in human metabolome might help with early unveiling of the pathological changes in disease processes.

Several studies have assessed the potential of metabolomics in diagnosis of DKD (Table 4). Han *et al.*<sup>[122]</sup> described the diverse profiles of plasma fatty acids in different stages of DKD. In 82 patients with T2DM, Zhu *et al.*<sup>[123]</sup> demonstrated that a panel of six plasma phospholipids was capable of distinguishing between patients with and without DKD. In 78 patients with diabetes, Hirayama *et al.*<sup>[124]</sup> identified a panel of 19 serum metabolites correlated significantly with UACR. A multiple logistic regression model composed of the five best performing markers (including γ-butyrobetaine, symmetric dimethylarginine, azelaic acid, and two unknowns) yielded remarkable sensitivity and specificity for the diagnosis of DKD. Sharma *et al.*<sup>[125]</sup> quantified 94 metabolites in urine obtained from healthy control, diabetic patients with and without DKD. A decrease in the urine levels of 13 metabolites, many potentially related to mitochondrial function, was found to be associated with DKD. Pena *et al.*<sup>[126]</sup> described the different metabolomic profiles in the urine and plasma samples from the T2DM cohort of the PREVEND study. Differences were observed in the levels of plasma histidine, butenoylcarnitine, as well as urine hexose, glutamine, and tyrosine, between those who with and without albuminuria. Adding these metabolites to a predictive model composed of baseline urinary albumin excretion and eGFR improve risk estimation for the progression of albuminuria. In the T2DM cohort of the Joslin Kidney Study, Niewczas *et al.*<sup>[127]</sup> identified a panel of 5 plasma metabolites capable of predicting progression toward ESRD, which was independent of UACR, eGFR, and hemoglobin A<sub>1c</sub>. Although



**Table 3** Urinary and serum miRNA profiles in patients with diabetic kidney disease

Type of diabetes	Specimen	miRNA expression	Ref.
1	Urine	Decreased miR-323b-5p, miR-221-3p, miR-524-5p, miR-188-3p	[109]
		Increased miR-214-3p, miR-92b-5p, hsa-miR-765, hsa-miR-429, miR-373-5p, miR-1913, miR-638	
1	Urine	Decreased miR-155, miR-424	[110]
2	Urine	miR-29 expression positively correlated to the severity of albuminuria	[111]
2	Blood	Reduced expression of miR-let-7a	[112]

**Table 4** Applications of metabolomics in the diagnosis of diabetic kidney disease

Specimen	Panel	Application	Ref.
Plasma	Fatty acids C10:0, C12:0, C14:0, C16:1n-9, C16:0, C18:2, C18:1n-9, C18:1n-11, C18:0, C20:4, C20:5, C20:3, C20:2, C20:0, C22:6	Diverse profiles in different stages of DKD	[122]
Plasma	Phospholipids C18:2-LPC, C16:0/18:1-PE, pC18:0/20:4-PE, C18:0/22:6-PI, C18:0/18:0-PS, dC18:0/20:2-SM	Diagnosis of DKD	[123]
Serum	$\gamma$ -butyrobetaine, SDMA, azelaic acid, MID 114, MID 127	Diagnosis of DKD	[124]
Urine	3-hydroxy isovalerate, aconitic acid, citric acid, 2-ethyl 3-OH propionate, glycolic acid, homovanillic acid, 3-hydroxy isobutyrate, 2-methyl acetoacetate, 3-methyl adipic acid, 3-methyl crotonyl glycine, 3-hydroxy propionate, tiglylglycine, uracil	Reduced expression in DKD patients	[125]
Plasma and urine	Plasma: Histidine, butenoylcarnitine Urine: Hexose, glutamine, tyrosine	Addition to the original predictive model improved risk estimation for albuminuria progression	[126]
Plasma	P-cresol sulfate, phenylacetylglutamine, myoinositol, pseudouridine, urate	Predicting progression toward ESRD	[127]

LPC: Lysophosphatidylcholine; PE: Phosphatidylethanolamine; PI: Phosphatidylinositol; PS: Phosphatidylserine; SM: Sphingomyelin; SDMA: Symmetric dimethylarginine; MID: Metabolite ID; DKD: Diabetic kidney disease; ESRD: End-stage renal disease.

these results seems promising, the complexity of the analysis techniques and the incomplete coverage of the human metabolome at present are problems than may need to be addressed before the application of metabolomics in everyday practice.

## CONCLUSION

The development of DKD involves the dysfunction and damage of different renal tissues in multiple stages. Due to the complex nature of this disease, whether there is a "universal" biomarker is questionable. With extensive validations, albuminuria and eGFR are currently the standard diagnostic criteria for DKD. Nonetheless, the abilities of these markers to detect tissue damage and functional change in the early stage are limited. With the increasing understanding of pathogenesis and promising preliminary data, applying the information generated from the studies of novel biomarkers, genomic, and proteomic profiles to assist in the early diagnosis of DKD has gradually become plausible. An integration of the "traditional" and "next-generation" markers might be more practical in everyday settings, considering the financial and technical requirements of these novel assays. To sum up, large longitudinal cohort studies are still required to validate the abilities of the aforementioned novel early diagnosis and prediction techniques.

## REFERENCES

- 1 **International Diabetes Federation.** IDF Diabetes Atlas, 7th ed. Brussels, Belgium: International Diabetes Federation, 2015
- 2 **Tuttle KR, Bakris GL, Bilous RW, Chiang JL, de Boer IH, Goldstein-Fuchs J, Hirsch IB, Kalantar-Zadeh K, Narva AS, Navaneethan SD, Neumiller JJ, Patel UD, Ratner RE, Whaley-Connell AT, Molitch ME.** Diabetic kidney disease: a report from an ADA Consensus Conference. *Diabetes Care* 2014; **37**: 2864-2883 [PMID: 25249672 DOI: 10.2337/dc14-1296]
- 3 **United States Renal Data System.** 2015 USRDS annual data report: Epidemiology of kidney disease in the United States. National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2015
- 4 **ERA-EDTA Registry.** ERA-EDTA Registry Annual Report 2013. Academic Medical Center, Department of Medical Informatics, Amsterdam, The Netherlands, 2015
- 5 **Wu MS, Wu IW, Shih CP, Hsu KH.** Establishing a Platform for Battling End-stage Renal Disease and Continuing Quality Improvement in Dialysis Therapy in Taiwan- Taiwan Renal Registry Data System (TWRDS). *Acta Nephrologica* 2011; **25**: 148-153. Available from: URL: <http://www.tsn.org.tw/tsnFile/journal/catalog/D8CE4CABE4354AB6/148-153.pdf>
- 6 **American Diabetes Association.** 9. Microvascular Complications and Foot Care. *Diabetes Care* 2016; **39** Suppl 1: S72-S80 [PMID: 26696685 DOI: 10.2337/dc16-S012]
- 7 **Helal I, Fick-Brosnahan GM, Reed-Gitomer B, Schrier RW.** Glomerular hyperfiltration: definitions, mechanisms and clinical implications. *Nat Rev Nephrol* 2012; **8**: 293-300 [PMID: 22349487 DOI: 10.1038/nrneph.2012.19]
- 8 **Espinell E, Agraz I, Ibernón M, Ramos N, Fort J, Serón D.** Renal Biopsy in Type 2 Diabetic Patients. *J Clin Med* 2015; **4**: 998-1009 [PMID: 26239461 DOI: 10.3390/jcm4050998]

- 9 **Ritz E**, Orth SR. Nephropathy in patients with type 2 diabetes mellitus. *N Engl J Med* 1999; **341**: 1127-1133 [PMID: 10511612 DOI: 10.1056/nejm199910073411506]
- 10 **Remuzzi G**, Schieppati A, Ruggenti P. Clinical practice. Nephropathy in patients with type 2 diabetes. *N Engl J Med* 2002; **346**: 1145-1151 [PMID: 11948275 DOI: 10.1056/NEJMcp011773]
- 11 **Levey AS**, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med* 1999; **130**: 461-470 [PMID: 10075613 DOI: 10.7326/0003-4819-130-6-199903160-00002]
- 12 **Levey AS**, Stevens LA, Schmid CH, Zhang YL, Castro AF, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J. A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009; **150**: 604-612 [PMID: 19414839 DOI: 10.7326/0003-4819-150-9-200905050-00006]
- 13 **Levey AS**, Coresh J, Balk E, Kausz AT, Levin A, Steffes MW, Hogg RJ, Perrone RD, Lau J, Eknoyan G. National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Ann Intern Med* 2003; **139**: 137-147 [PMID: 12859163 DOI: 10.7326/0003-4819-139-2-200307150-00013]
- 14 **Perrone RD**, Madias NE, Levey AS. Serum creatinine as an index of renal function: new insights into old concepts. *Clin Chem* 1992; **38**: 1933-1953 [PMID: 1394976]
- 15 **Baxmann AC**, Ahmed MS, Marques NC, Menon VB, Pereira AB, Kirsztajn GM, Heilberg IP. Influence of muscle mass and physical activity on serum and urinary creatinine and serum cystatin C. *Clin J Am Soc Nephrol* 2008; **3**: 348-354 [PMID: 18235143 DOI: 10.2215/cjn.02870707]
- 16 **Rule AD**, Larson TS, Bergstralh EJ, Slezak JM, Jacobsen SJ, Cosio FG. Using serum creatinine to estimate glomerular filtration rate: accuracy in good health and in chronic kidney disease. *Ann Intern Med* 2004; **141**: 929-937 [PMID: 15611490 DOI: 10.7326/0003-4819-141-12-200412210-00009]
- 17 **Levey AS**, Coresh J, Greene T, Stevens LA, Zhang YL, Hendriksen S, Kusek JW, Van Lente F. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Ann Intern Med* 2006; **145**: 247-254 [PMID: 16908915]
- 18 **Michels WM**, Grootendorst DC, Verduijn M, Elliott EG, Dekker FW, Krediet RT. Performance of the Cockcroft-Gault, MDRD, and new CKD-EPI formulas in relation to GFR, age, and body size. *Clin J Am Soc Nephrol* 2010; **5**: 1003-1009 [PMID: 20299365 DOI: 10.2215/cjn.06870909]
- 19 **Camargo EG**, Soares AA, Detanico AB, Weinert LS, Veronese FV, Gomes EC, Silveiro SP. The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation is less accurate in patients with Type 2 diabetes when compared with healthy individuals. *Diabet Med* 2011; **28**: 90-95 [PMID: 21166850 DOI: 10.1111/j.1464-5491.2010.03161.x]
- 20 **Keen H**, Chlouverakis C. An immunoassay method for urinary albumin at low concentrations. *Lancet* 1963; **2**: 913-914 [PMID: 14052063]
- 21 **Eknoyan G**, Hostetter T, Bakris GL, Hebert L, Levey AS, Parving HH, Steffes MW, Toto R. Proteinuria and other markers of chronic kidney disease: a position statement of the national kidney foundation (NKF) and the national institute of diabetes and digestive and kidney diseases (NIDDK). *Am J Kidney Dis* 2003; **42**: 617-622 [PMID: 14520612]
- 22 **National Kidney Foundation**. KDOQI Clinical Practice Guidelines and Clinical Practice Recommendations for Diabetes and Chronic Kidney Disease. *Am J Kidney Dis* 2007; **49**: S12-S154 [PMID: 17276798 DOI: 10.1053/j.ajkd.2006.12.005]
- 23 **Perkins BA**, Ficociello LH, Roshan B, Warram JH, Krolewski AS. In patients with type 1 diabetes and new-onset microalbuminuria the development of advanced chronic kidney disease may not require progression to proteinuria. *Kidney Int* 2010; **77**: 57-64 [PMID: 19847154 DOI: 10.1038/ki.2009.399]
- 24 **Garg AX**, Kiberd BA, Clark WF, Haynes RB, Clase CM. Albuminuria and renal insufficiency prevalence guides population screening: results from the NHANES III. *Kidney Int* 2002; **61**: 2165-2175 [PMID: 12028457 DOI: 10.1046/j.1523-1755.2002.00356.x]
- 25 **Kramer HJ**, Nguyen QD, Curhan G, Hsu CY. Renal insufficiency in the absence of albuminuria and retinopathy among adults with type 2 diabetes mellitus. *JAMA* 2003; **289**: 3273-3277 [PMID: 12824208 DOI: 10.1001/jama.289.24.3273]
- 26 **Retnakaran R**, Cull CA, Thorne KI, Adler AI, Holman RR. Risk factors for renal dysfunction in type 2 diabetes: U.K. Prospective Diabetes Study 74. *Diabetes* 2006; **55**: 1832-1839 [PMID: 16731850 DOI: 10.2337/db05-1620]
- 27 **Dwyer JP**, Parving HH, Hunsicker LG, Ravid M, Remuzzi G, Lewis JB. Renal Dysfunction in the Presence of Normoalbuminuria in Type 2 Diabetes: Results from the DEMAND Study. *Cardiorenal Med* 2012; **2**: 1-10 [PMID: 22493597 DOI: 10.1159/000333249]
- 28 **Mauer SM**, Steffes MW, Ellis EN, Sutherland DE, Brown DM, Goetz FC. Structural-functional relationships in diabetic nephropathy. *J Clin Invest* 1984; **74**: 1143-1155 [PMID: 6480821 DOI: 10.1172/jci11523]
- 29 **Caramori ML**, Kim Y, Huang C, Fish AJ, Rich SS, Miller ME, Russell G, Mauer M. Cellular basis of diabetic nephropathy: 1. Study design and renal structural-functional relationships in patients with long-standing type 1 diabetes. *Diabetes* 2002; **51**: 506-513 [PMID: 11812762 DOI: 10.2337/diabetes.51.2.506]
- 30 **Fioretto P**, Mauer M, Brocco E, Velussi M, Frigato F, Muollo B, Sambataro M, Abaterusso C, Baggio B, Crepaldi G, Nosadini R. Patterns of renal injury in NIDDM patients with microalbuminuria. *Diabetologia* 1996; **39**: 1569-1576 [PMID: 8960844 DOI: 10.1007/s001250050616]
- 31 **Brocco E**, Fioretto P, Mauer M, Saller A, Carraro A, Frigato F, Chiesura-Corona M, Bianchi L, Baggio B, Maioli M, Abaterusso C, Velussi M, Sambataro M, Virgili F, Ossi E, Nosadini R. Renal structure and function in non-insulin dependent diabetic patients with microalbuminuria. *Kidney Int Suppl* 1997; **63**: S40-S44 [PMID: 9407419]
- 32 **Dalla Vestra M**, Saller A, Bortoloso E, Mauer M, Fioretto P. Structural involvement in type 1 and type 2 diabetic nephropathy. *Diabetes Metab* 2000; **26** Suppl 4: 8-14 [PMID: 10922968]
- 33 **Penno G**, Solini A, Bonora E, Fondelli C, Orsi E, Zerbini G, Trevisan R, Vedovato M, Gruden G, Cavalot F, Cignarelli M, Laviola L, Morano S, Nicolucci A, Pugliese G. Clinical significance of nonalbuminuric renal impairment in type 2 diabetes. *J Hypertens* 2011; **29**: 1802-1809 [PMID: 21738053 DOI: 10.1097/HJH.0b013e3283495cd6]
- 34 **Ogawa Y**, Goto T, Tamasawa N, Matsui J, Tando Y, Sugimoto K, Tomotsune K, Kimura M, Yasujima M, Suda T. Serum cystatin C in diabetic patients. Not only an indicator for renal dysfunction in patients with overt nephropathy but also a predictor for cardiovascular events in patients without nephropathy. *Diabetes Res Clin Pract* 2008; **79**: 357-361 [PMID: 17980929 DOI: 10.1016/j.diabetes.2007.09.016]
- 35 **Kyhse-Andersen J**, Schmidt C, Nordin G, Andersson B, Nilsson-Ehle P, Lindström V, Grubb A. Serum cystatin C, determined by a rapid, automated particle-enhanced turbidimetric method, is a better marker than serum creatinine for glomerular filtration rate. *Clin Chem* 1994; **40**: 1921-1926 [PMID: 7923773]
- 36 **Finney H**, Newman DJ, Price CP. Adult reference ranges for serum cystatin C, creatinine and predicted creatinine clearance. *Ann Clin Biochem* 2000; **37** (Pt 1): 49-59 [PMID: 10672373 DOI: 10.1258/0004563001901524]
- 37 **Laterza OF**, Price CP, Scott MG. Cystatin C: an improved estimator of glomerular filtration rate? *Clin Chem* 2002; **48**: 699-707 [PMID: 11978596]
- 38 **Mussap M**, Dalla Vestra M, Fioretto P, Saller A, Varagnolo M, Nosadini R, Plebani M. Cystatin C is a more sensitive marker than creatinine for the estimation of GFR in type 2 diabetic patients. *Kidney Int* 2002; **61**: 1453-1461 [PMID: 11918752 DOI: 10.1046/j.1523-1755.2002.00253.x]
- 39 **Perkins BA**, Nelson RG, Ostrander BE, Blouch KL, Krolewski

- AS, Myers BD, Warram JH. Detection of renal function decline in patients with diabetes and normal or elevated GFR by serial measurements of serum cystatin C concentration: results of a 4-year follow-up study. *J Am Soc Nephrol* 2005; **16**: 1404-1412 [PMID: 15788478 DOI: 10.1681/asn.2004100854]
- 40 Goetz DH, Willie ST, Armen RS, Bratt T, Borregaard N, Strong RK. Ligand preference inferred from the structure of neutrophil gelatinase associated lipocalin. *Biochemistry* 2000; **39**: 1935-1941 [PMID: 10684642 DOI: 10.1021/bi992215v]
- 41 Bolignano D, Donato V, Coppolino G, Campo S, Buemi A, Lacquaniti A, Buemi M. Neutrophil gelatinase-associated lipocalin (NGAL) as a marker of kidney damage. *Am J Kidney Dis* 2008; **52**: 595-605 [PMID: 18725016 DOI: 10.1053/j.ajkd.2008.01.020]
- 42 Yang YH, He XJ, Chen SR, Wang L, Li EM, Xu LY. Changes of serum and urine neutrophil gelatinase-associated lipocalin in type-2 diabetic patients with nephropathy: one year observational follow-up study. *Endocrine* 2009; **36**: 45-51 [PMID: 19390997 DOI: 10.1007/s12020-009-9187-x]
- 43 Nielsen SE, Schjoedt KJ, Astrup AS, Tarnow L, Lajer M, Hansen PR, Parving HH, Rossing P. Neutrophil Gelatinase-Associated Lipocalin (NGAL) and Kidney Injury Molecule 1 (KIM1) in patients with diabetic nephropathy: a cross-sectional study and the effects of lisinopril. *Diabet Med* 2010; **27**: 1144-1150 [PMID: 20854382 DOI: 10.1111/j.1464-5491.2010.03083.x]
- 44 Fu WJ, Li BL, Wang SB, Chen ML, Deng RT, Ye CQ, Liu L, Fang AJ, Xiong SL, Wen S, Tang HH, Chen ZX, Huang ZH, Peng LF, Zheng L, Wang Q. Changes of the tubular markers in type 2 diabetes mellitus with glomerular hyperfiltration. *Diabetes Res Clin Pract* 2012; **95**: 105-109 [PMID: 22015481 DOI: 10.1016/j.diabres.2011.09.031]
- 45 van Timmeren MM, van den Heuvel MC, Bailly V, Bakker SJ, van Goor H, Stegeman CA. Tubular kidney injury molecule-1 (KIM-1) in human renal disease. *J Pathol* 2007; **212**: 209-217 [PMID: 17471468 DOI: 10.1002/path.2175]
- 46 Vaidya VS, Niewczas MA, Ficociello LH, Johnson AC, Collings FB, Warram JH, Krolewski AS, Bonventre JV. Regression of microalbuminuria in type 1 diabetes is associated with lower levels of urinary tubular injury biomarkers, kidney injury molecule-1, and N-acetyl- $\beta$ -D-glucosaminidase. *Kidney Int* 2011; **79**: 464-470 [PMID: 20980978 DOI: 10.1038/ki.2010.404]
- 47 Bazzi C, Petrini C, Rizza V, Arrigo G, Napodano P, Paparella M, D'Amico G. Urinary N-acetyl-beta-glucosaminidase excretion is a marker of tubular cell dysfunction and a predictor of outcome in primary glomerulonephritis. *Nephrol Dial Transplant* 2002; **17**: 1890-1896 [PMID: 12401843 DOI: 10.1093/ndt/17.11.1890]
- 48 Liangos O, Perianayagam MC, Vaidya VS, Han WK, Wald R, Tighiouart H, MacKinnon RW, Li L, Balakrishnan VS, Pereira BJ, Bonventre JV, Jaber BL. Urinary N-acetyl-beta-(D)-glucosaminidase activity and kidney injury molecule-1 level are associated with adverse outcomes in acute renal failure. *J Am Soc Nephrol* 2007; **18**: 904-912 [PMID: 17267747 DOI: 10.1681/asn.2006030221]
- 49 Widstam-Attorps U, Berg U. Urinary protein excretion and renal function in young people with diabetes mellitus. *Nephrol Dial Transplant* 1992; **7**: 487-492 [PMID: 1320227]
- 50 Uslu S, Efe B, Alataş O, Kebapçı N, Colak O, Demirüstü C, Yörük A. Serum cystatin C and urinary enzymes as screening markers of renal dysfunction in diabetic patients. *J Nephrol* 2005; **18**: 559-567 [PMID: 16299682]
- 51 Kern EF, Erhard P, Sun W, Genuth S, Weiss MF. Early urinary markers of diabetic kidney disease: a nested case-control study from the Diabetes Control and Complications Trial (DCCT). *Am J Kidney Dis* 2010; **55**: 824-834 [PMID: 20138413 DOI: 10.1053/j.ajkd.2009.11.009]
- 52 Hong CY, Chia KS, Ling SL. Urinary protein excretion in Type 2 diabetes with complications. *J Diabetes Complications* 2000; **14**: 259-265 [PMID: 11113688 DOI: 10.1016/S1056-8727(00)00119-7]
- 53 Weitgasser R, Schnoell F, Gappmayer B, Kartnig I. Prospective evaluation of urinary N-acetyl-beta-D-glucosaminidase with respect to macrovascular disease in elderly type 2 diabetic patients. *Diabetes Care* 1999; **22**: 1882-1886 [PMID: 10546024 DOI: 10.2337/diacare.22.11.1882]
- 54 Yoshikawa R, Wada J, Seiki K, Matsuoka T, Miyamoto S, Takahashi K, Ota S, Taniaki K, Hida K, Yamakado M, Shikata K, Uehara Y, Urade Y, Makino H. Urinary PGDS levels are associated with vascular injury in type 2 diabetes patients. *Diabetes Res Clin Pract* 2007; **76**: 358-367 [PMID: 17007955 DOI: 10.1016/j.diabres.2006.09.004]
- 55 Macisaac RJ, Ekinci EI, Jerums G. Markers of and risk factors for the development and progression of diabetic kidney disease. *Am J Kidney Dis* 2014; **63**: S39-S62 [PMID: 24461729 DOI: 10.1053/j.ajkd.2013.10.048]
- 56 Cooke MS, Evans MD, Herbert KE, Lunec J. Urinary 8-oxo-2'-deoxyguanosine--source, significance and supplements. *Free Radic Res* 2000; **32**: 381-397 [PMID: 10766407 DOI: 10.1080/1071576000300391]
- 57 Wu LL, Chiou CC, Chang PY, Wu JT. Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetes. *Clin Chim Acta* 2004; **339**: 1-9 [PMID: 14687888]
- 58 Hinokio Y, Suzuki S, Hirai M, Chiba M, Hirai A, Toyota T. Oxidative DNA damage in diabetes mellitus: its association with diabetic complications. *Diabetologia* 1999; **42**: 995-998 [PMID: 10491760 DOI: 10.1007/s001250051258]
- 59 Hinokio Y, Suzuki S, Hirai M, Suzuki C, Suzuki M, Toyota T. Urinary excretion of 8-oxo-7, 8-dihydro-2'-deoxyguanosine as a predictor of the development of diabetic nephropathy. *Diabetologia* 2002; **45**: 877-882 [PMID: 12107732 DOI: 10.1007/s00125-002-0831-8]
- 60 Brownlee M. Glycation products and the pathogenesis of diabetic complications. *Diabetes Care* 1992; **15**: 1835-1843 [PMID: 1464241]
- 61 Price DL, Rhett PM, Thorpe SR, Baynes JW. Chelating activity of advanced glycation end-product inhibitors. *J Biol Chem* 2001; **276**: 48967-48972 [PMID: 11677237 DOI: 10.1074/jbc.M108196200]
- 62 Calabrese V, Mancuso C, Sapienza M, Puleo E, Calafato S, Cornelius C, Finocchiaro M, Mangiameli A, Di Mauro M, Stella AM, Castellino P. Oxidative stress and cellular stress response in diabetic nephropathy. *Cell Stress Chaperones* 2007; **12**: 299-306 [PMID: 18229449]
- 63 Piarulli F, Sartore G, Ceriello A, Ragazzi E, Reitano R, Nollino L, Cosma C, Fedele D, Lapolla A. Relationship between glyco-oxidation, antioxidant status and microalbuminuria in type 2 diabetic patients. *Diabetologia* 2009; **52**: 1419-1425 [PMID: 19401824 DOI: 10.1007/s00125-009-1367-y]
- 64 Niewczas MA, Ficociello LH, Johnson AC, Walker W, Rosolowsky ET, Roshan B, Warram JH, Krolewski AS. Serum concentrations of markers of TNFalpha and Fas-mediated pathways and renal function in nonproteinuric patients with type 1 diabetes. *Clin J Am Soc Nephrol* 2009; **4**: 62-70 [PMID: 19073786 DOI: 10.2215/cjn.03010608]
- 65 Gohda T, Niewczas MA, Ficociello LH, Walker WH, Skupien J, Rosetti F, Cullere X, Johnson AC, Crabtree G, Smiles AM, Mayadas TN, Warram JH, Krolewski AS. Circulating TNF receptors 1 and 2 predict stage 3 CKD in type 1 diabetes. *J Am Soc Nephrol* 2012; **23**: 516-524 [PMID: 22266664 DOI: 10.1681/asn.2011060628]
- 66 Niewczas MA, Gohda T, Skupien J, Smiles AM, Walker WH, Rosetti F, Cullere X, Eckfeldt JH, Doria A, Mayadas TN, Warram JH, Krolewski AS. Circulating TNF receptors 1 and 2 predict ESRD in type 2 diabetes. *J Am Soc Nephrol* 2012; **23**: 507-515 [PMID: 22266663 DOI: 10.1681/asn.2011060627]
- 67 Forsblom CM, Kanninen T, Lehtovirta M, Saloranta C, Groop LC. Heritability of albumin excretion rate in families of patients with Type II diabetes. *Diabetologia* 1999; **42**: 1359-1366 [PMID: 10550421 DOI: 10.1007/s001250051450]
- 68 Fogarty DG, Rich SS, Hanna L, Warram JH, Krolewski AS. Urinary albumin excretion in families with type 2 diabetes is heritable and genetically correlated to blood pressure. *Kidney Int* 2000; **57**: 250-257 [PMID: 10620206 DOI: 10.1046/j.1523-1755.2000.00833.x]
- 69 Langefeld CD, Beck SR, Bowden DW, Rich SS, Wagenknecht LE, Freedman BI. Heritability of GFR and albuminuria in Caucasians



- with type 2 diabetes mellitus. *Am J Kidney Dis* 2004; **43**: 796-800 [PMID: 15112169]
- 70 **Vardarli I**, Baier LJ, Hanson RL, Akkoyun I, Fischer C, Rohmeiss P, Basci A, Bartram CR, Van Der Woude FJ, Janssen B. Gene for susceptibility to diabetic nephropathy in type 2 diabetes maps to 18q22.3-23. *Kidney Int* 2002; **62**: 2176-2183 [PMID: 12427143 DOI: 10.1046/j.1523-1755.2002.00663.x]
  - 71 **Bowden DW**, Colicigno CJ, Langefeld CD, Sale MM, Williams A, Anderson PJ, Rich SS, Freedman BI. A genome scan for diabetic nephropathy in African Americans. *Kidney Int* 2004; **66**: 1517-1526 [PMID: 15458446 DOI: 10.1111/j.1523-1755.2004.00915.x]
  - 72 **Shimazaki A**, Kawamura Y, Kanazawa A, Sekine A, Saito S, Tsunoda T, Koya D, Babazono T, Tanaka Y, Matsuda M, Kawai K, Iizumi T, Imanishi M, Shinosaki T, Yanagimoto T, Ikeda M, Omachi S, Kashiwagi A, Kaku K, Iwamoto Y, Kawamori R, Kikkawa R, Nakajima M, Nakamura Y, Maeda S. Genetic variations in the gene encoding ELMO1 are associated with susceptibility to diabetic nephropathy. *Diabetes* 2005; **54**: 1171-1178 [PMID: 15793258]
  - 73 **Pezzolesi MG**, Katavetin P, Kure M, Poznik GD, Skupien J, Mychaleckyj JC, Rich SS, Warram JH, Krolewski AS. Confirmation of genetic associations at ELMO1 in the GoKinD collection supports its role as a susceptibility gene in diabetic nephropathy. *Diabetes* 2009; **58**: 2698-2702 [PMID: 19651817 DOI: 10.2337/db09-0641]
  - 74 **Leak TS**, Perlegas PS, Smith SG, Keene KL, Hicks PJ, Langefeld CD, Mychaleckyj JC, Rich SS, Kirk JK, Freedman BI, Bowden DW, Sale MM. Variants in intron 13 of the ELMO1 gene are associated with diabetic nephropathy in African Americans. *Ann Hum Genet* 2009; **73**: 152-159 [PMID: 19183347 DOI: 10.1111/j.1469-1809.2008.00498.x]
  - 75 **Craig DW**, Millis MP, DiStefano JK. Genome-wide SNP genotyping study using pooled DNA to identify candidate markers mediating susceptibility to end-stage renal disease attributed to Type 1 diabetes. *Diabet Med* 2009; **26**: 1090-1098 [PMID: 19929986 DOI: 10.1111/j.1464-5491.2009.02846.x]
  - 76 **Williams WW**, Salem RM, McKnight AJ, Sandholm N, Forsblom C, Taylor A, Guiducci C, McAteer JB, McKay GJ, Isakova T, Brennan EP, Sadlier DM, Palmer C, Söderlund J, Fagerholm E, Harjutsalo V, Lithovius R, Gordin D, Hietala K, Kytö J, Parkkonen M, Rosengård-Bärlund M, Thorn L, Syreeni A, Tolonen N, Saraheimo M, Wadén J, Pitkäniemi J, Sarti C, Tuomilehto J, Tryggvason K, Österholm AM, He B, Bain S, Martin F, Godson C, Hirschhorn JN, Maxwell AP, Groop PH, Florez JC. Association testing of previously reported variants in a large case-control meta-analysis of diabetic nephropathy. *Diabetes* 2012; **61**: 2187-2194 [PMID: 22721967 DOI: 10.2337/db11-0751]
  - 77 **Wu HY**, Wang Y, Chen M, Zhang X, Wang D, Pan Y, Li L, Liu D, Dai XM. Association of ELMO1 gene polymorphisms with diabetic nephropathy in Chinese population. *J Endocrinol Invest* 2013; **36**: 298-302 [PMID: 22842811 DOI: 10.3275/8525]
  - 78 **Pezzolesi MG**, Poznik GD, Mychaleckyj JC, Paterson AD, Barati MT, Klein JB, Ng DP, Placha G, Canani LH, Bochenski J, Waggott D, Merchant ML, Krolewski B, Mirea L, Wanic K, Katavetin P, Kure M, Wolkow P, Dunn JS, Smiles A, Walker WH, Boright AP, Bull SB, Doria A, Rogus JJ, Rich SS, Warram JH, Krolewski AS. Genome-wide association scan for diabetic nephropathy susceptibility genes in type 1 diabetes. *Diabetes* 2009; **58**: 1403-1410 [PMID: 19252134 DOI: 10.2337/db08-1514]
  - 79 **Maeda S**, Araki S, Babazono T, Toyoda M, Umezono T, Kawai K, Imanishi M, Uzu T, Watada H, Suzuki D, Kashiwagi A, Iwamoto Y, Kaku K, Kawamori R, Nakamura Y. Replication study for the association between four Loci identified by a genome-wide association study on European American subjects with type 1 diabetes and susceptibility to diabetic nephropathy in Japanese subjects with type 2 diabetes. *Diabetes* 2010; **59**: 2075-2079 [PMID: 20460425 DOI: 10.2337/db10-0067]
  - 80 **Park H**, Kim HJ, Lee S, Yoo YJ, Ju YS, Lee JE, Cho SI, Sung J, Kim JI, Seo JS. A family-based association study after genome-wide linkage analysis identified two genetic loci for renal function in a Mongolian population. *Kidney Int* 2013; **83**: 285-292 [PMID: 23254893 DOI: 10.1038/ki.2012.389]
  - 81 **Pezzolesi MG**, Jeong J, Smiles AM, Skupien J, Mychaleckyj JC, Rich SS, Warram JH, Krolewski AS. Family-based association analysis confirms the role of the chromosome 9q21.32 locus in the susceptibility of diabetic nephropathy. *PLoS One* 2013; **8**: e60301 [PMID: 23555951 DOI: 10.1371/journal.pone.0060301]
  - 82 **McDonough CW**, Palmer ND, Hicks PJ, Roh BH, An SS, Cooke JN, Hester JM, Wing MR, Bostrom MA, Rudock ME, Lewis JP, Talbert ME, Blevins RA, Lu L, Ng MC, Sale MM, Divers J, Langefeld CD, Freedman BI, Bowden DW. A genome-wide association study for diabetic nephropathy genes in African Americans. *Kidney Int* 2011; **79**: 563-572 [PMID: 21150874 DOI: 10.1038/ki.2010.467]
  - 83 **Cooke JN**, Bostrom MA, Hicks PJ, Ng MC, Hellwege JN, Comeau ME, Divers J, Langefeld CD, Freedman BI, Bowden DW. Polymorphisms in MYH9 are associated with diabetic nephropathy in European Americans. *Nephrol Dial Transplant* 2012; **27**: 1505-1511 [PMID: 21968013 DOI: 10.1093/ndt/gfr522]
  - 84 **Chang YC**, Chang EY, Chuang LM. Recent progress in the genetics of diabetic microvascular complications. *World J Diabetes* 2015; **6**: 715-725 [PMID: 26069720 DOI: 10.4239/wjd.v6.i5.715]
  - 85 **Conserva F**, Gesualdo L, Papale M. A Systems Biology Overview on Human Diabetic Nephropathy: From Genetic Susceptibility to Post-Transcriptional and Post-Translational Modifications. *J Diabetes Res* 2016; **2016**: 7934504 [PMID: 26798653 DOI: 10.1155/2016/7934504]
  - 86 **Richter K**, Konzack A, Pihlajaniemi T, Heljasvaara R, Kietzmann T. Redox-fibrosis: Impact of TGFβ1 on ROS generators, mediators and functional consequences. *Redox Biol* 2015; **6**: 344-352 [PMID: 26335400 DOI: 10.1016/j.redox.2015.08.015]
  - 87 **Pirola L**, Balcerzyk A, Okabe J, El-Osta A. Epigenetic phenomena linked to diabetic complications. *Nat Rev Endocrinol* 2010; **6**: 665-675 [PMID: 21045787 DOI: 10.1038/nrendo.2010.188]
  - 88 **Hasegawa K**, Wakino S, Simic P, Sakamaki Y, Minakuchi H, Fujimura K, Hosoya K, Komatsu M, Kaneko Y, Kanda T, Kubota E, Tokuyama H, Hayashi K, Guarente L, Itoh H. Renal tubular Sirt1 attenuates diabetic albuminuria by epigenetically suppressing Claudin-1 overexpression in podocytes. *Nat Med* 2013; **19**: 1496-1504 [PMID: 24141423 DOI: 10.1038/nm.3363]
  - 89 **Bell CG**, Teschendorff AE, Rakyen VK, Maxwell AP, Beck S, Savage DA. Genome-wide DNA methylation analysis for diabetic nephropathy in type 1 diabetes mellitus. *BMC Med Genomics* 2010; **3**: 33 [PMID: 20687937 DOI: 10.1186/1755-8794-3-33]
  - 90 **Sapienza C**, Lee J, Powell J, Erinle O, Yafai F, Reichert J, Siraj ES, Madaio M. DNA methylation profiling identifies epigenetic differences between diabetes patients with ESRD and diabetes patients without nephropathy. *Epigenetics* 2011; **6**: 20-28 [PMID: 21150313]
  - 91 **Chua JH**, Armugam A, Jeyaseelan K. MicroRNAs: biogenesis, function and applications. *Curr Opin Mol Ther* 2009; **11**: 189-199 [PMID: 19330724]
  - 92 **Mitchell PS**, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, Peterson A, Noteboom J, O'Brian KC, Allen A, Lin DW, Urban N, Drescher CW, Knudsen BS, Stirewalt DL, Gentleman R, Vessella RL, Nelson PS, Martin DB, Tewari M. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci USA* 2008; **105**: 10513-10518 [PMID: 18663219 DOI: 10.1073/pnas.0804549105]
  - 93 **Schena FP**, Sallustio F, Serino G. microRNAs in glomerular diseases from pathophysiology to potential treatment target. *Clin Sci (Lond)* 2015; **128**: 775-788 [PMID: 25881669 DOI: 10.1042/cs20140733]
  - 94 **Kato M**, Zhang J, Wang M, Lanting L, Yuan H, Rossi JJ, Natarajan R. MicroRNA-192 in diabetic kidney glomeruli and its function in TGF-beta-induced collagen expression via inhibition of E-box repressors. *Proc Natl Acad Sci USA* 2007; **104**: 3432-3437 [PMID: 17360662 DOI: 10.1073/pnas.0611192104]
  - 95 **Wang B**, Herman-Edelstein M, Koh P, Burns W, Jandeleit-Dahm K, Watson A, Saleem M, Goodall GJ, Twigg SM, Cooper ME,



- Kantharidis P. E-cadherin expression is regulated by miR-192/215 by a mechanism that is independent of the profibrotic effects of transforming growth factor-beta. *Diabetes* 2010; **59**: 1794-1802 [PMID: 20393144 DOI: 10.2337/db09-1736]
- 96 **Putta S**, Lanting L, Sun G, Lawson G, Kato M, Natarajan R. Inhibiting microRNA-192 ameliorates renal fibrosis in diabetic nephropathy. *J Am Soc Nephrol* 2012; **23**: 458-469 [PMID: 22223877 DOI: 10.1681/asn.2011050485]
- 97 **Krupa A**, Jenkins R, Luo DD, Lewis A, Phillips A, Fraser D. Loss of MicroRNA-192 promotes fibrogenesis in diabetic nephropathy. *J Am Soc Nephrol* 2010; **21**: 438-447 [PMID: 20056746 DOI: 10.1681/asn.2009050530]
- 98 **Kato M**, Wang L, Putta S, Wang M, Yuan H, Sun G, Lanting L, Todorov I, Rossi JJ, Natarajan R. Post-transcriptional up-regulation of Tsc-22 by Ybx1, a target of miR-216a, mediates TGF- $\beta$ -induced collagen expression in kidney cells. *J Biol Chem* 2010; **285**: 34004-34015 [PMID: 20713358 DOI: 10.1074/jbc.M110.165027]
- 99 **Wang Q**, Wang Y, Minto AW, Wang J, Shi Q, Li X, Quigg RJ. MicroRNA-377 is up-regulated and can lead to increased fibronectin production in diabetic nephropathy. *FASEB J* 2008; **22**: 4126-4135 [PMID: 18716028 DOI: 10.1096/fj.08-112326]
- 100 **Long J**, Wang Y, Wang W, Chang BH, Danesh FR. MicroRNA-29c is a signature microRNA under high glucose conditions that targets Sprouty homolog 1, and its in vivo knockdown prevents progression of diabetic nephropathy. *J Biol Chem* 2011; **286**: 11837-11848 [PMID: 21310958 DOI: 10.1074/jbc.M110.194969]
- 101 **Kato M**, Arce L, Wang M, Putta S, Lanting L, Natarajan R. A microRNA circuit mediates transforming growth factor- $\beta$ 1 auto-regulation in renal glomerular mesangial cells. *Kidney Int* 2011; **80**: 358-368 [PMID: 21389977 DOI: 10.1038/ki.2011.43]
- 102 **Wang JY**, Gao YB, Zhang N, Zou DW, Wang P, Zhu ZY, Li JY, Zhou SN, Wang SC, Wang YY, Yang JK. miR-21 overexpression enhances TGF- $\beta$ 1-induced epithelial-to-mesenchymal transition by target smad7 and aggravates renal damage in diabetic nephropathy. *Mol Cell Endocrinol* 2014; **392**: 163-172 [PMID: 24887517 DOI: 10.1016/j.mce.2014.05.018]
- 103 **Alvarez ML**, Khosroheidari M, Eddy E, Kiefer J. Role of microRNA 1207-5P and its host gene, the long non-coding RNA Pvt1, as mediators of extracellular matrix accumulation in the kidney: implications for diabetic nephropathy. *PLoS One* 2013; **8**: e77468 [PMID: 24204837 DOI: 10.1371/journal.pone.0077468]
- 104 **Wang B**, Koh P, Winbanks K, Coughlan MT, McClelland A, Watson A, Jandeleit-Dahm K, Burns WC, Thomas MC, Cooper ME, Kantharidis P. miR-200a Prevents renal fibrogenesis through repression of TGF- $\beta$ 2 expression. *Diabetes* 2011; **60**: 280-287 [PMID: 20952520 DOI: 10.2337/db10-0892]
- 105 **Zhao B**, Li H, Liu J, Han P, Zhang C, Bai H, Yuan X, Wang X, Li L, Ma H, Jin X, Chu Y. MicroRNA-23b Targets Ras GTPase-Activating Protein SH3 Domain-Binding Protein 2 to Alleviate Fibrosis and Albuminuria in Diabetic Nephropathy. *J Am Soc Nephrol* 2016; Epub ahead of print [PMID: 26839366 DOI: 10.1681/asn.2015030300]
- 106 **Long J**, Wang Y, Wang W, Chang BH, Danesh FR. Identification of microRNA-93 as a novel regulator of vascular endothelial growth factor in hyperglycemic conditions. *J Biol Chem* 2010; **285**: 23457-23465 [PMID: 20501654 DOI: 10.1074/jbc.M110.136168]
- 107 **Fu Y**, Zhang Y, Wang Z, Wang L, Wei X, Zhang B, Wen Z, Fang H, Pang Q, Yi F. Regulation of NADPH oxidase activity is associated with miRNA-25-mediated NOX4 expression in experimental diabetic nephropathy. *Am J Nephrol* 2010; **32**: 581-589 [PMID: 21071935 DOI: 10.1159/000322105]
- 108 **Zhang Z**, Luo X, Ding S, Chen J, Chen T, Chen X, Zha H, Yao L, He X, Peng H. MicroRNA-451 regulates p38 MAPK signaling by targeting of Ywhaz and suppresses the mesangial hypertrophy in early diabetic nephropathy. *FEBS Lett* 2012; **586**: 20-26 [PMID: 21827757 DOI: 10.1016/j.febslet.2011.07.042]
- 109 **Argyropoulos C**, Wang K, McClarty S, Huang D, Bernardo J, Ellis D, Orchard T, Galas D, Johnson J. Urinary microRNA profiling in the nephropathy of type 1 diabetes. *PLoS One* 2013; **8**: e54662 [PMID: 23358711 DOI: 10.1371/journal.pone.0054662]
- 110 **Barutta F**, Tricarico M, Corbelli A, Annaratone L, Pinach S, Grimaldi S, Bruno G, Cimino D, Taverna D, Derigibus MC, Rastaldi MP, Perin PC, Gruden G. Urinary exosomal microRNAs in incipient diabetic nephropathy. *PLoS One* 2013; **8**: e73798 [PMID: 24223694 DOI: 10.1371/journal.pone.0073798]
- 111 **Peng H**, Zhong M, Zhao W, Wang C, Zhang J, Liu X, Li Y, Paudel SD, Wang Q, Lou T. Urinary miR-29 correlates with albuminuria and carotid intima-media thickness in type 2 diabetes patients. *PLoS One* 2013; **8**: e82607 [PMID: 24349318 DOI: 10.1371/journal.pone.0082607]
- 112 **Zhou J**, Peng R, Li T, Luo X, Peng H, Zha H, Yin P, Wen L, Zhang Z. A potentially functional polymorphism in the regulatory region of let-7a-2 is associated with an increased risk for diabetic nephropathy. *Gene* 2013; **527**: 456-461 [PMID: 23860321 DOI: 10.1016/j.gene.2013.06.088]
- 113 **Kenyon GL**, DeMarini DM, Fuchs E, Galas DJ, Kirsch JF, Leyh TS, Moos WH, Petsko GA, Ringe D, Rubin GM, Sheahan LC. Defining the mandate of proteomics in the post-genomics era: workshop report. *Mol Cell Proteomics* 2002; **1**: 763-780 [PMID: 12438560]
- 114 **Currie G**, McKay G, Delles C. Biomarkers in diabetic nephropathy: Present and future. *World J Diabetes* 2014; **5**: 763-776 [PMID: 25512779 DOI: 10.4239/wjd.v5.i6.763]
- 115 **Rossing K**, Mischak H, Dakna M, Zürgbig P, Novak J, Julian BA, Good DM, Coon JJ, Tarnow L, Rossing P. Urinary proteomics in diabetes and CKD. *J Am Soc Nephrol* 2008; **19**: 1283-1290 [PMID: 18448586 DOI: 10.1681/asn.2007091025]
- 116 **Good DM**, Zürgbig P, Argilés A, Bauer HW, Behrens G, Coon JJ, Dakna M, Decramer S, Delles C, Dominiczak AF, Ehrlich JH, Eitner F, Fliser D, Frommberger M, Ganser A, Girolami MA, Golovko I, Gwinner W, Haubitz M, Herget-Rosenthal S, Jankowski J, Jahn H, Jerums G, Julian BA, Kellmann M, Kliem V, Kolch W, Krolewski AS, Luppi M, Masy Z, Melter M, Neustüss C, Novak J, Peter K, Rossing K, Rupperecht H, Schanstra JP, Schiffer E, Stolzenburg JU, Tarnow L, Theodorescu D, Thongboonkerd V, Vanholder R, Weissinger EM, Mischak H, Schmitt-Kopplin P. Naturally occurring human urinary peptides for use in diagnosis of chronic kidney disease. *Mol Cell Proteomics* 2010; **9**: 2424-2437 [PMID: 20616184 DOI: 10.1074/mcp.M110.001917]
- 117 **Zürgbig P**, Jerums G, Hovind P, Macisaac RJ, Mischak H, Nielsen SE, Panagiotopoulos S, Persson F, Rossing P. Urinary proteomics for early diagnosis in diabetic nephropathy. *Diabetes* 2012; **61**: 3304-3313 [PMID: 22872235 DOI: 10.2337/db12-0348]
- 118 **Roscioni SS**, de Zeeuw D, Hellemons ME, Mischak H, Zürgbig P, Bakker SJ, Gansevoort RT, Reinhard H, Persson F, Lajer M, Rossing P, Lambers Heerspink HJ. A urinary peptide biomarker set predicts worsening of albuminuria in type 2 diabetes mellitus. *Diabetologia* 2013; **56**: 259-267 [PMID: 23086559 DOI: 10.1007/s00125-012-2755-2]
- 119 **Siwy J**, Schanstra JP, Argiles A, Bakker SJ, Beige J, Boucek P, Brand K, Delles C, Duranton F, Fernandez-Fernandez B, Jankowski ML, Al Khatib M, Kunt T, Lajer M, Lichtinghagen R, Lindhardt M, Maahs DM, Mischak H, Mullen W, Navis G, Noutsou M, Ortiz A, Persson F, Petrie JR, Roob JM, Rossing P, Ruggenti P, Rychlik I, Serra AL, Snell-Bergeon J, Spasovski G, Stojceva-Taneva O, Trillini M, von der Leyen H, Winkhofer-Roob BM, Zürgbig P, Jankowski J. Multicentre prospective validation of a urinary peptidome-based classifier for the diagnosis of type 2 diabetic nephropathy. *Nephrol Dial Transplant* 2014; **29**: 1563-1570 [PMID: 24589724 DOI: 10.1093/ndt/gfu039]
- 120 **Lindon JC**, Holmes E, Bollard ME, Stanley EG, Nicholson JK. Metabonomics technologies and their applications in physiological monitoring, drug safety assessment and disease diagnosis. *Biomarkers* 2004; **9**: 1-31 [PMID: 15204308 DOI: 10.1080/13547500410001668379]
- 121 **Wishart DS**, Knox C, Guo AC, Eisner R, Young N, Gautam B, Hau DD, Psychogios N, Dong E, Bouatra S, Mandal R, Sinelnikov I, Xia J, Jia L, Cruz JA, Lim E, Sobsey CA, Shrivastava S, Huang P, Liu P, Fang L, Peng J, Fradette R, Cheng D, Tzur D, Clements M, Lewis A, De Souza A, Zuniga A, Dawe M, Xiong Y, Clive D, Greiner R, Nazzyrova A, Shaykhutdinov R, Li L, Vogel HJ, Forsythe

- I. HMDB: a knowledgebase for the human metabolome. *Nucleic Acids Res* 2009; **37**: D603-D610 [PMID: 18953024 DOI: 10.1093/nar/gkn810]
- 122 **Han LD**, Xia JF, Liang QL, Wang Y, Wang YM, Hu P, Li P, Luo GA. Plasma esterified and non-esterified fatty acids metabolic profiling using gas chromatography-mass spectrometry and its application in the study of diabetic mellitus and diabetic nephropathy. *Anal Chim Acta* 2011; **689**: 85-91 [PMID: 21338761 DOI: 10.1016/j.aca.2011.01.034]
- 123 **Zhu C**, Liang QL, Hu P, Wang YM, Luo GA. Phospholipidomic identification of potential plasma biomarkers associated with type 2 diabetes mellitus and diabetic nephropathy. *Talanta* 2011; **85**: 1711-1720 [PMID: 21872008 DOI: 10.1016/j.talanta.2011.05.036]
- 124 **Hirayama A**, Nakashima E, Sugimoto M, Akiyama S, Sato W, Maruyama S, Matsuo S, Tomita M, Yuzawa Y, Soga T. Metabolic profiling reveals new serum biomarkers for differentiating diabetic nephropathy. *Anal Bioanal Chem* 2012; **404**: 3101-3109 [PMID: 23052862 DOI: 10.1007/s00216-012-6412-x]
- 125 **Sharma K**, Karl B, Mathew AV, Gangoiiti JA, Wassel CL, Saito R, Pu M, Sharma S, You YH, Wang L, Diamond-Stanic M, Lindenmeyer MT, Forsblom C, Wu W, Ix JH, Ideker T, Kopp JB, Nigam SK, Cohen CD, Groop PH, Barshop BA, Natarajan L, Nyhan WL, Naviaux RK. Metabolomics reveals signature of mitochondrial dysfunction in diabetic kidney disease. *J Am Soc Nephrol* 2013; **24**: 1901-1912 [PMID: 23949796 DOI: 10.1681/asn.2013020126]
- 126 **Pena MJ**, Lambers Heerspink HJ, Hellemons ME, Friedrich T, Dallmann G, Lajer M, Bakker SJ, Gansevoort RT, Rossing P, de Zeeuw D, Roscioni SS. Urine and plasma metabolites predict the development of diabetic nephropathy in individuals with Type 2 diabetes mellitus. *Diabet Med* 2014; **31**: 1138-1147 [PMID: 24661264 DOI: 10.1111/dme.12447]
- 127 **Niewczas MA**, Sirich TL, Mathew AV, Skupien J, Mohny RP, Warram JH, Smiles A, Huang X, Walker W, Byun J, Karoly ED, Kensicki EM, Berry GT, Bonventre JV, Pennathur S, Meyer TW, Krolewski AS. Uremic solutes and risk of end-stage renal disease in type 2 diabetes: metabolomic study. *Kidney Int* 2014; **85**: 1214-1224 [PMID: 24429397 DOI: 10.1038/ki.2013.497]

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Editorial Board Member of *World Journal of Diabetes*, Joshua J Neumiller, Pharm D, Associate Professor, Department of Pharmacotherapy, College of Pharmacy, Washington State University, Spokane, WA 99210-1495, United States

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*WJD* covers topics concerning  $\alpha$ ,  $\beta$ ,  $\delta$  and PP cells of the pancreatic islet, the effect of insulin and insulinresistance, pancreatic islet transplantation, adipose cells and obesity.

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*World Journal of Diabetes*

Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China

Telephone: +86-10-85381891

Fax: +86-10-85381893

E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)

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## Pharmacogenetic studies update in type 2 diabetes mellitus

Shalini Singh, Kauser Usman, Monisha Banerjee

Shalini Singh, Monisha Banerjee, Molecular and Human Genetics Laboratory, Department of Zoology, University of Lucknow, Lucknow 226007, India

Kauser Usman, Department of Medicine, King George's Medical University, Lucknow 226003, India

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**Correspondence to:** Dr. Monisha Banerjee, Professor, Molecular and Human Genetics Laboratory, Department of Zoology, University of Lucknow, University Road, Lucknow 226007, India. [banerjee\\_monisha30@rediffmail.com](mailto:banerjee_monisha30@rediffmail.com)  
 Telephone: +91-98-39500439

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### Abstract

Type 2 diabetes mellitus (T2DM) is a silent progressive polygenic metabolic disorder resulting from ineffective insulin cascading in the body. World-wide, about 415 million people are suffering from T2DM with a projected

rise to 642 million in 2040. T2DM is treated with several classes of oral antidiabetic drugs (OADs) viz. biguanides, sulfonylureas, thiazolidinediones, meglitinides, etc. Treatment strategies for T2DM are to minimize long-term micro and macro vascular complications by achieving an optimized glycemic control. Genetic variations in the human genome not only disclose the risk of T2DM development but also predict the personalized response to drug therapy. Inter-individual variability in response to OADs is due to polymorphisms in genes encoding drug receptors, transporters, and metabolizing enzymes for example, genetic variants in solute carrier transporters (*SLC22A1*, *SLC22A2*, *SLC22A3*, *SLC47A1* and *SLC47A2*) are actively involved in glycemic/HbA1c management of metformin. In addition, *CYP* gene encoding Cytochrome P450 enzymes also play a crucial role with respect to metabolism of drugs. Pharmacogenetic studies provide insights on the relationship between individual genetic variants and variable therapeutic outcomes of various OADs. Clinical utility of pharmacogenetic study is to predict the therapeutic dose of various OADs on individual basis. Pharmacogenetics therefore, is a step towards personalized medicine which will greatly improve the efficacy of diabetes treatment.

**Key words:** Type 2 diabetes mellitus; Pharmacogenetics; Genetic variants; Oral antidiabetic drugs; Personalized medicine

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**Core tip:** Type 2 diabetes mellitus (T2DM) is a highly prevalent metabolic disorder, characterized by chronic hyperglycemia. It results from an interaction of environmental as well as genetic factors. Several genes have been identified associated with disease development and therapeutic outcomes. Inter-individual variations in the human genome affect both, risk of T2DM development and personalized response to identical drug therapies. Pharmacogenetic approaches focus on single nucleotide polymorphisms and their influence on individual drug response, efficacy and toxicity. In the

present study, an effort has been made to review the genetic polymorphisms in candidate genes associated with efficacy of oral antidiabetic drugs.

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## INTRODUCTION

Type 2 diabetes mellitus (T2DM) has been considered as a major health problem for both developed as well as developing countries. The global burden of diabetes is presently 415 million affected people, expected to rise to 642 million in 2040 and about 193 million people still undiagnosed. The Indian estimate is also alarming which shows 69.2 million people affected with T2DM in 2015 which will rise to 123.5 million in 2040<sup>[1]</sup>.

Diabetes is traditionally known as a “silent disease” manifesting no symptoms until it progresses to severe damage of target organs. Diabetes has been classified under various categories depending upon their age of onset and severity<sup>[2]</sup>. The most prevalent adult-onset diabetes is T2DM characterized by hyperglycemia caused by defects in both insulin secretion and insulin signaling cascade. T2DM is a potential contributor to considerable morbidity in the form of metabolic complications viz. heart disease, stroke, neuropathy, kidney disease, vision disorder, peripheral vascular disease, ulcerations and amputations, infection, digestive diseases, oral complications and depression. T2DM is a multifactorial disease with high genetic variability in which certain candidate genes interfere with management of glycemic control in the body. Polymorphisms in the candidate genes may affect the susceptibility or risk of disease development and progression<sup>[3-7]</sup>.

Pharmacogenomics establishes the use of an individual's genetic information to guide treatment therapy and has become an important tool in achieving “personalized medicine”. The discoveries of novel genetic polymorphisms in drug transporters, and metabolizing enzymes have given an insight into the biological phenomena of drug efficacy and toxicity (Figure 1). Pharmacologically, several classes of drugs are currently being prescribed to treat T2DM patients, including biguanides, sulfonylureas, meglitinides, thiazolidinediones (TZDs),  $\alpha$ -Glucosidase inhibitors, dipeptidyl peptidase-4 (DPP-4) inhibitors, glucagon-like peptide-1 (GLP-1) agonist, sodium-glucose co-transporter-2 inhibitors, insulin and its analogues<sup>[8-10]</sup>. Clinically, it is often observed that T2DM patients who receive identical antidiabetic regimens often exhibit significant variation in glycemic control, glycated haemoglobin (HbA1c) level, drug efficacy, tolerability and incidence

of adverse effects<sup>[11]</sup>. Inter-individual differences can be attributed to polymorphisms of certain candidate genes involved in drug absorption, transportation, distribution, metabolism and signaling cascade of oral antidiabetic drugs (OADs)<sup>[11]</sup>.

## PHARMACOGENETICS

The term “pharmacogenetics” was coined by Vogel *et al*<sup>[12]</sup> which explains the differential response of individuals to identical medication. Clinical observations of inherited inter-individual differences during treatment were documented for the first time in 1950s<sup>[13-15]</sup> giving rise to a new field, *i.e.*, pharmacogenetics and later pharmacogenomics. Pharmacogenomics is being used for genome-wide approaches to recognize the inherited inter-individual differences in response to drugs. Pharmacogenetics reveals that single nucleotide variations in genes (encoding drug receptor, transporters and metabolizing enzymes) are related to the efficacy and toxicity of drugs<sup>[16-18]</sup>, for example *CYP2D6*, *CYP2C8* and *CYP2C9* are marked *CYP* enzymes that are actively involved in metabolism of various therapeutic agents<sup>[19]</sup>.

The inter-individual differences are contributed by numerous factors, *i.e.*, physical inactivity, race/ethnic diversity, hypertension, age, gender, *etc*<sup>[20]</sup>. During past decades, pharmacogenetic study was restricted to observations of familial response to a particular drug. However, genome-wide association studies, candidate gene approach and linkage analysis have transformed the area of pharmacogenetics/pharmacogenomics. These studies have elucidated the role of genetic variations for a particular drug and their doses on a personalized basis.

## PHARMACOGENETICS OF T2DM IN PROSPECT WITH OADS

Treatment strategy for T2DM is mainly based on efficacy of OADs assessed by level of fasting/postprandial plasma glucose and/or HbA1c<sup>[10]</sup>.

## BIGUANIDES

Metformin (N',N'-dimethylbiguanide) is prescribed as a first-line medication for newly diagnosed T2DM patients<sup>[21]</sup>. Antihyperglycemic effects of metformin includes down regulation of hepatic gluconeogenesis, improvement in insulin sensitivity and significant reduction in insulin resistance<sup>[22]</sup>. The precise mechanism(s) of metformin action are still not fully elucidated. At physiological pH metformin serves as an organic cation being transported across the membrane by different isoforms of organic cation transporters (OCTs) viz. OCT1 expressed in hepatocytes, OCT2 in basolateral membrane of kidney. Metformin is transported from intestinal lumen into the epithelial cells *via* OCT3 and plasma membrane monoamine transporter. Uptake of metformin from blood into hepatocytes is mediated

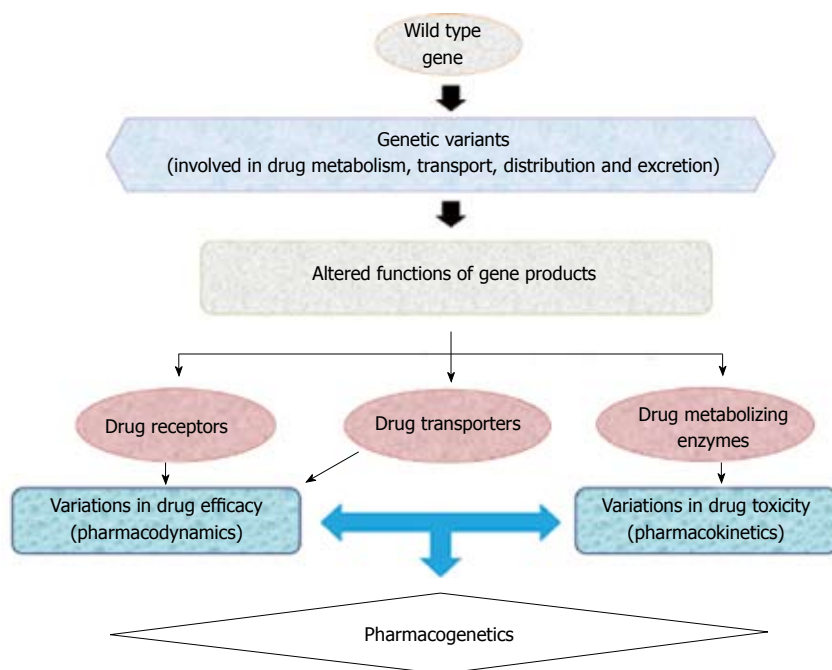


Figure 1 Effects of gene polymorphisms on drug efficacy and toxicity.

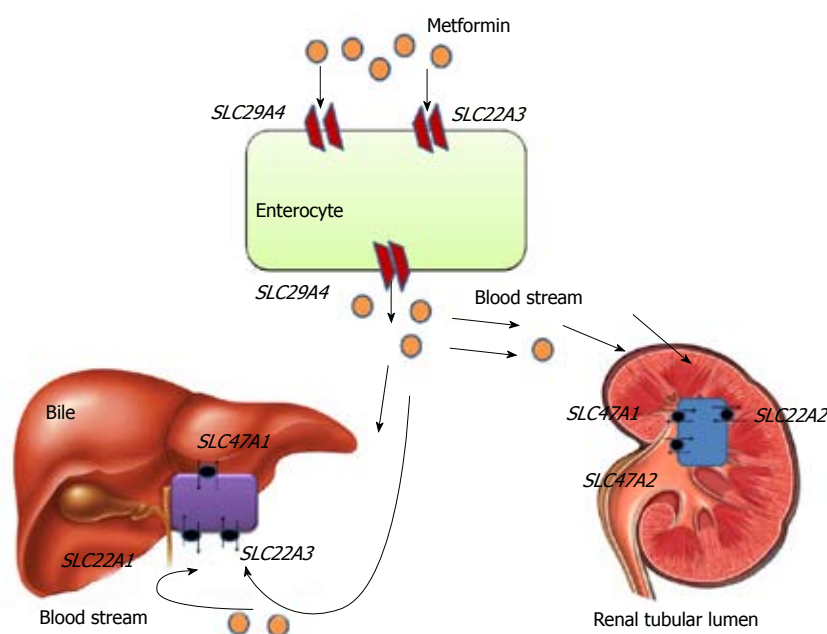


Figure 2 Schematic representation of cellular locations of metformin transporters. SLC22A3: Solute carrier family 22 member 3; SLC29A4: Solute carrier family 29 member 4.

by OCT1 and OCT3 (Figure 2). Metformin interferes with mitochondrial respiratory chain complex 1 by increasing AMP/ATP ratio, which promotes the activation of AMP kinase<sup>[23,24]</sup>. Metformin-induced AMP kinase activation leads to transcriptional inhibition of hepatic gluconeogenesis<sup>[25]</sup>. Metformin is not metabolized and excreted-out through urine *via* active renal tubular secretion. Metformin excretion in bile and urine is also facilitated by various isoforms of Multidrug and Toxin Extrusion transporters (MATE1 and MATE2)<sup>[26,27]</sup>. Therapeutic response of metformin differs inter-individually due to genetic polymorphisms. Single nucleotide polymorphisms (SNPs) in the genes encoding metformin transporters *viz.* OCT1, OCT2, MATE1, MATE2, *etc.*, leads to significant association with the

different degrees of efficacy and toxicity (Figure 2).

## GENES ASSOCIATED WITH BIGUANIDE TREATMENT

### Solute carrier family 22 member 1

Solute carrier family 22 member 1 (*SLC22A1*) gene encodes the OCT1 which is expressed in hepatocytes and mediates the electrogenic transport of drugs<sup>[28]</sup>. OCT1 helps in transport of metformin into the liver (hepatocytes) and subsequent activity. It has been hypothesized that highly polymorphic *SLC22A1* gene will influence the therapeutic success rate of metformin. In a South Indian study<sup>[29]</sup>, it was reported that rs622342

variant of *SLC22A1* gene was significantly associated with efficacy of metformin. They found that T2DM patients with rs622342 "AA" homozygotes had 5.6 times increased possibility of responding to metformin treatment. A recent pharmacogenetic study performed in a Chinese population demonstrated that T2DM patients with "AA" genotype of *SLC22A1* rs594709 might have maximum plasma glucose lowering effect from metformin monotherapy<sup>[30]</sup>. Shu *et al.*<sup>[31]</sup> studied the effect of loss of function polymorphism in *SLC22A1* gene variants, *i.e.*, rs12208357 (R61C), rs34130495 (G401S), rs72552763 (420del), rs34059508 (G465R). They concluded the study as these variants were significantly associated with lower efficacy of metformin in glucose tolerance test. However, in a subsequent GoDARTs study, two common *SLC22A1* variants, R61C (rs12208357) and 420del (rs72552763) were reported to have no association with impaired initial response to metformin, or metformin monotherapy failure<sup>[32]</sup>.

### Solute carrier family 22 member 2

Solute carrier family 22 member 2 (*SLC22A2*) gene encodes the OCT2. OCT2 is a drug transporter and expressed in renal tubular cells thought to be responsible for their elimination<sup>[33,34]</sup>. Loss of function mutation in *SLC22A2* gene has been significantly correlated with metformin disposition. In several studies, *SLC22A2* gene has been reported as highly polymorphic in nature<sup>[34-37]</sup>. Zolk *et al.*<sup>[38]</sup> found that *SLC22A2* variant 808G > T (270Ala > Ser) significantly transforms the uptake of drugs. In healthy subjects, rs316019 (A270S) variant appeared responsible for decreased renal clearance of Metformin<sup>[30]</sup> while in a contradictory study a significant correlation of rs316019 was reported with increased metformin renal clearance<sup>[39]</sup>. Song *et al.*<sup>[40]</sup> investigated the influence of rs201919874 (T199I) and rs145450955 (T201M) to the disposition of metformin in healthy individuals and reported that both were significantly associated with increased metformin plasma concentration and reduced renal clearance. A recent randomized cohort study performed in T2DM patients with one year follow-up demonstrated that efficacy of metformin was also influenced by *SLC22A2* variant, rs316019 (808G > T)<sup>[41]</sup>.

### Solute carrier family 22 member 3

Solute carrier family 22 member 3 (*SLC22A3*) gene encodes for OCT3 which is expressed in liver, kidney and placenta. In public SNP database (<http://www.ncbi.nlm.nih.gov/SNP/>) five non-synonymous variants (ssj0008476, rs8187717, rs8187725, rs12212246, rs9365165) of human *SLC22A3* gene were reported<sup>[42]</sup>. However, compared to OCT1 and OCT2, very few studies have reported about OCT3 variants and metformin therapeutics. In a pharmacologic study, Chen *et al.*<sup>[43]</sup> studied the role of OCT3 variants ssj0008476 (T44M), rs8187725 (T400I) and V423F were found to be significantly associated with altered response to metformin action.

### Solute carrier family 47 member 1

Solute carrier family 47 member 1 (*SLC47A1*) gene encodes the multidrug toxin extrusion receptor 1 expressed on apical domain of proximal and distal renal tubular cells and serves as an electro neutral organic cation/H<sup>+</sup> exchanger. Since genetic polymorphisms in *SLC47A1* associated with altered transport/excretion function might have great influence on metformin disposition, it is important to identify them in various ethnic populations and correlate in terms of therapeutic response. An intronic variant rs2289669 (G > A) in *SLC47A1* was demonstrated to reduce HbA1c level significantly in metformin users<sup>[44]</sup>. While in a DPP (Diabetes Prevention Programme) study, *SLC47A1* variant rs8065082 (C > T) was reported for lower diabetes incidence in individuals treated with metformin<sup>[45]</sup>. In a recent case control study the polymorphic effect of rs594709 in *SLC22A1* and rs2289669 in *SLC47A1* was evaluated in T2DM cases and no significant association was reported. The study concluded that carriers of allele "A" of rs594709 showed better efficacy for metformin<sup>[30]</sup>. In Chinese population, the *SLC47A1* variant rs2289669 (G > A) appeared to promote metformin efficacy by delaying its excretion mechanism<sup>[46]</sup>.

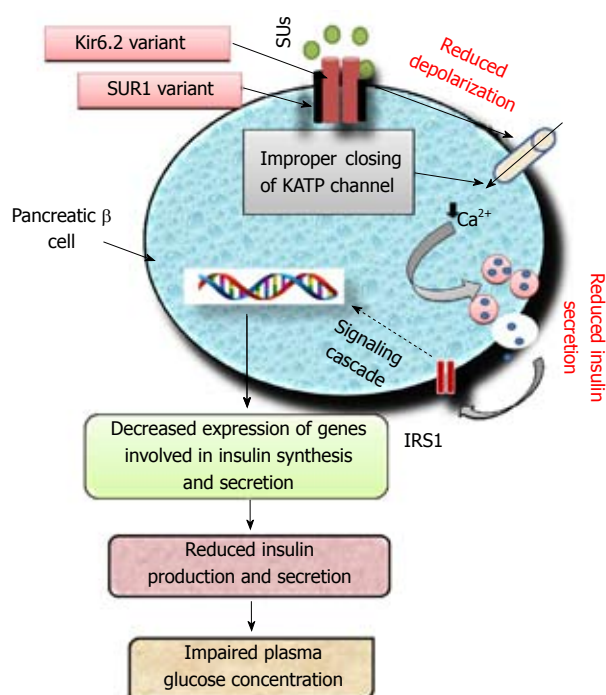
### Solute carrier family 47 member 2

Solute carrier family 47 member 2 (*SLC47A2*) encodes for multidrug toxin extrusion receptor 2 (MATE2), expressed in apical membrane proximal tubule cells. It facilitates the disposition of metformin from renal tubular cells into urine. Choi *et al.*<sup>[47]</sup> characterized variants of *SLC47A2* to recognize their association with metformin. The study showed that homozygous individuals for rs12943590 (130G > A) of MATE2-K is significantly associated with poor plasma glucose control of metformin assessed by relative differences in HbA1C level.

## SULFONYLUREAS

Sulfonylureas (SUs), insulin secretagogues are one of the most common classes of OADs being prescribed either alone or in combination since 1960s<sup>[8,48]</sup>. The second generation drugs *viz.* glimepiride, glibenclamide (glyburide), gliclazide and glipizide are most common representatives belonging to the group of SUs. The first-generation drugs *viz.* tolbutamide and chlorpropamide are no longer prescribed<sup>[10]</sup>. All SUs stimulate insulin secretion by binding to sulfonylurea receptor 1 (SUR1), a protein having 1581-amino acids. This interaction depolarizes the cell membrane of pancreatic beta cells by closing ATP-sensitive potassium (KATP) channels. Subsequent effect of depolarization leads to Ca<sup>2+</sup> influx which trigger an enhanced insulin secretion from beta cells in glucose-independent manner<sup>[49]</sup>. KATP channel is a heterooctameric protein complex constructed by four inward-rectifier K<sup>+</sup> channel, *i.e.*, Kir6.2 (forming pore of KATP channel) coupled with SUR1, surrounding the pore<sup>[49]</sup>. In neonates, inactivating mutations in genes encoding Kir6.2 (*KCNJ11*) and SUR1 (*ABCC8*)





**Figure 3** Schematic diagram showing the Kir6.2 and SUR1 variants affecting sulfonylurea efficacy. Pancreatic β cell membrane with SUR1/Kir6.2 variant leads to improper closing of KATP channel on binding with SUs. This subsequently leads to poor membrane depolarization and less influx of Ca<sup>2+</sup> ions which will result in less and delayed insulin secretion. Hence, low level of insulin molecules will be available to bind with IRS1 and lead to an impaired signaling cascade resulting in poor management of glycemic condition. SUs: Sulfonylureas; SUR1: Sulfonylurea receptor 1; KATP: ATP-sensitive potassium channel; IRS1: Insulin receptor substrate 1; SUR 1: Sulfonylurea Receptor 1.

are responsible for T2DM, while activating mutations lead to hypoglycemia<sup>[50]</sup>. Polymorphisms in the genes *ABCC8*, *KCNJ11*, *CYP2C9*, *TCF7L2*, *NOS1AP* (nitric oxide synthase 1 adaptor protein) have been reported for altered response to SUs<sup>[51,52]</sup>. Impairment of Kir6.2 (*KCNJ11*) and SUR1 (*ABCC8*) will lead to improper signaling cascade of insulin as shown in Figure 3.

## GENES ASSOCIATED WITH SULFONYLUREA TREATMENT

### Potassium inwardly-rectifying channel, subfamily J, member 11

ATP-sensitive potassium channel (KATP) is a trans-membrane protein of pancreatic β-cells encoded by potassium inwardly-rectifying channel and subfamily J, member 11 (*KCNJ11*). Two hundred and nineteen SNPs have been reported for the *KCNJ11* gene located on chromosome 11p15.1. Polymorphisms in *KCNJ11* have been reported for development of diabetes because of its key role in insulin secretion<sup>[53]</sup>. Only 6 SNPs viz. rs5210, rs5215, rs5218, rs5219, rs886288, rs2285676 have been reported to be associated with diabetes<sup>[54]</sup>. A study found that in T2DM patients the rs5210 variant located at 3' UTR of *KCNJ11* improves the clinical efficacy of glizalide<sup>[52]</sup>. The most widely studied *KCNJ11* gene variant rs5219 (E23K) was significantly associated

with the onset of T2DM in Asian Indian and Chinese populations<sup>[55,56]</sup>. However, studies performed on Caucasian individuals demonstrated for no significant differences in glycated hemoglobin<sup>[57,58]</sup>. Some studies have reported that diabetic patients having *KCNJ11* gene variants respond better to pharmacotherapy with SUs as compared to insulin<sup>[59-61]</sup>.

### ATP-binding cassette, subfamily C member 8

ATP-binding cassette, subfamily C member 8 (*ABCC8*) located at 11p15.1, encodes for SUR1 which modulate the activity of KATP channel<sup>[62]</sup>. Variants of *ABCC8* gene rs1799854 (C/T) and rs1801261 have been studied extensively and are reported for inconsistent association with T2DM<sup>[63-70]</sup>. *ABCC8* variant rs1799854 has been reported for significant association with sulfonylurea efficacy in terms of HbA1c level<sup>[57]</sup>. In one study, the genetic variants of *ABCC8* were reported for significant reduction in HbA1c concentration<sup>[71]</sup>. Activating mutation in the genes encoding SUR1 (*ABCC8*) and Kir6.2 (*KCNJ11*) may lead to altered signaling cascade of insulin secretagogues resulting in therapeutic failure of SUs. The Arg972 variant of insulin receptor substrate 1 is reported for an enhanced risk of secondary failure to SUs in T2DM patients<sup>[72]</sup>. A study carried out in Chinese T2DM patients with two months follow-up, demonstrated that Ser1369Ala variant of *ABCC8* is significantly associated with therapeutic success of glizalide<sup>[73]</sup>. Carriers and non-carriers of SUR1-437A/T variant did not differ in insulin response stimulated by tolbutamide during OGT test<sup>[74]</sup>.

### Cytochrome P450 2C9

SUs viz. tolbutamide, glimiperide, glipizide and glibenclamide are metabolized to active metabolites in the liver mainly by cytochrome P450 2C9 (*CYP2C9*)<sup>[75]</sup> which are ultimately excreted by the kidney<sup>[76]</sup>. It has been reported that *CYP2C9* variants were significantly associated with efficacy of SUs in diabetic patients<sup>[77]</sup>. Two variants of *CYP2C9* gene, i.e., rs1057910 (*CYP2C9*\*3) and rs1799853 (*CYP2C9*\*2) have been significantly associated with missense amino acid polymorphisms resulting in decreased metabolism of SUs in healthy volunteers<sup>[74]</sup>. While in T2DM patients treated with SUs, *CYP2C9*\*3 variant was reported with an enhanced risk of severe hypoglycemia<sup>[78,79]</sup>. Certain T2DM patients with *CYP2C9* gene variants Ile359Leu and Arg144Cys were reported for 30%-80% reduction in renal clearance of glibenclamide suggesting lower doses of this antidiabetic drug to decrease the risk of hypoglycemia<sup>[51,75,77,80,81]</sup>.

### Transcription factor 7-like 2

Transcription factor 7-like 2 (*TCF7L2*) is encoded by *TCF7L2* gene which is actively involved in proliferation and differentiation of cells. It is required for secretion of glucose stimulated insulin from pancreatic β-cells. *TCF7L2* is a key transcription factor, which regulates glucose metabolism in insulin dependent manner. It serves as a chief regulator in coordinating the proinsulin

synthesis and its processing to produce mature insulin<sup>[82]</sup>. Hence, nucleotide variation in *TCF7L2* gene may lead to alteration in insulin secretion<sup>[31]</sup> resulting reduced insulin secretion will lead to hyperglycemia. *TCF7L2* gene is expressed in developing and mature pancreatic beta cells<sup>[83]</sup> and secretion of insulin is decreased in individuals having risk alleles<sup>[84-86]</sup>. Miyake *et al.*<sup>[87]</sup> studied association of *TCF7L2* variants with susceptibility to T2DM in 4087 Japanese patients. They found that rs7903146, rs12255372 and rs11196205 were significantly associated with T2DM while rs290487 and rs11196218 were reported for no association. Polymorphisms in *TCF7L2* gene has been reported for strong association with T2DM affecting therapeutic response to SUs<sup>[88]</sup>. *TCF7L2* SNPs were reported to influence the risk of T2DM<sup>[89]</sup>. Polymorphisms in *TCF7L2*, rs12255372 and rs7903146 were reported for decreased response to sulfonylurea efficacy<sup>[88]</sup>. The SNPs rs12255372T and rs7903146T were represented to be significantly associated with enhanced expression of *TCF7L2* gene in beta cells, altering insulin release and predisposing individuals to T2DM<sup>[90,91]</sup>.

## TZDS

TZDs are insulin sensitizers, they promote uptake of glucose by tissue and skeletal muscles, down regulate glucose output from liver<sup>[92]</sup>. Rosiglitazone and pioglitazone are medical representatives of TZD group. The exact molecular mechanism of TZDs is far from clear. However, data indicates that TZDs primarily bind with the peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) in adipose tissue and affect their metabolism. On binding with PPAR $\gamma$ , TZDs stimulate adipocytes differentiation<sup>[93]</sup> and decrease plasma glucose level in T2DM patients<sup>[94,95]</sup>. Several studies have reported that TZDs improve both glucose homeostasis and insulin cascading in T2DM cases<sup>[96-98]</sup>, hence may prevent the progression from altered plasma glucose tolerance to T2DM development<sup>[99]</sup>. Numerous potential mechanisms are reported by which TZDs improve molecular action of insulin in both liver<sup>[98,100]</sup> and skeletal muscles<sup>[97,101]</sup>. These include reduced content of intra-hepatocellular and intra-myocellular triglycerides<sup>[98,102]</sup> and altered body composition<sup>[97,103]</sup>. It also decreases synthesis and/or action of proinflammatory cytokines<sup>[104,105]</sup>. TZDs upregulate expression of genes in adipocytes resulting in increased level of adiponectin in plasma circulation<sup>[106-108]</sup>, with positive effects on insulin sensitivity<sup>[109]</sup> and reduced hyperglycemia. Some previous studies reported that use of rosiglitazone (a TZD drug) as compared to pioglitazone could cause severe side effects, the risk of myocardial infarction and also lead to death due to cardiovascular dysfunction<sup>[110,111]</sup>. Several gene variants have been identified for significant association with therapeutic outcome of TZDs. Adiponectin, resistin, leptin, TNF- $\alpha$  and PPAR $\gamma$  are commonly called adipocytokines which are key regulators of insulin resistance<sup>[112]</sup>.

## GENES ASSOCIATED WITH TZD

### TREATMENT

#### PPAR $\gamma$

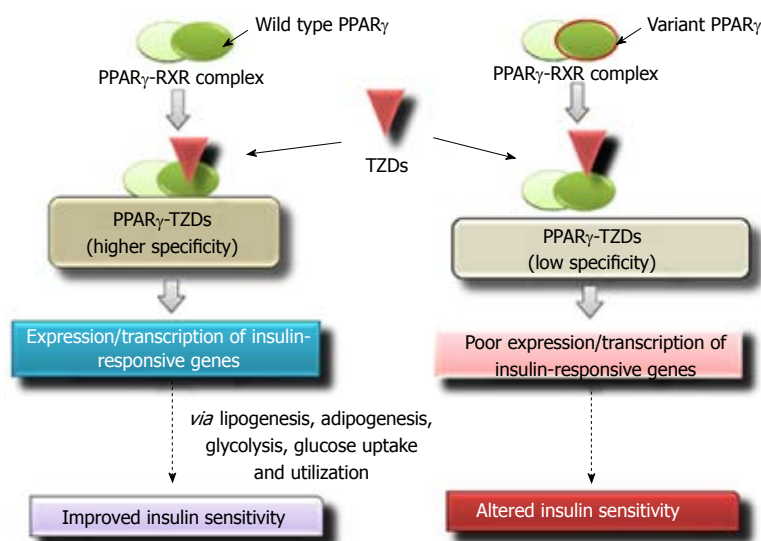
PPAR $\gamma$  belonging to the nuclear receptor family regulates metabolism of carbohydrates, regulates lipid homeostasis and adipocyte differentiation<sup>[113]</sup>. It is also a key mediator of insulin signaling<sup>[114]</sup>. In humans, TZDs bind to PPAR $\gamma$  with high specificity. At physiological pH PPAR $\gamma$  forms a dimer with retinoid X receptor (RXR). Binding of TZDs to the PPAR $\gamma$ -RXR complex stimulates a conformational change<sup>[115]</sup> which subsequently leads to the binding of the above heterodimer complex to the PPAR $\gamma$  response elements (PPRE) in the target genes<sup>[116]</sup>. It results in improved insulin sensitivity *via* glycolysis, lipogenesis, adipogenesis and increased glucose uptake and utilization<sup>[117]</sup>. Single nucleotide variations in PPAR $\gamma$  gene may affect the binding affinity with TZDs and its therapeutic efficacy (Figure 4). In PPAR $\gamma$  gene, loss-of-function mutations are significantly associated with insulin resistance and T2DM<sup>[118]</sup>. Multiple studies have reported that missense polymorphism Pro12Ala (CCA-to-GCA) in PPAR $\gamma$  gene is associated with decreased risk of T2DM development<sup>[119-121]</sup> and improved insulin sensitivity<sup>[122,123]</sup>. A pilot study performed on South Indian population, evaluated the effect of Pro12Ala variants on therapeutic success to pioglitazone, and reported a significant association with glycemic control<sup>[124]</sup>. T2DM cases with Pro12Ala variant of PPAR $\gamma$  gene, showed significant glycemic control [fasting plasma glucose (FPG) and HbA1c level] for rosiglitazone treatment as compared with carriers having wild-type genotype<sup>[125]</sup>. Zhang *et al.*<sup>[126]</sup> demonstrated that in Chinese patients, amino acid variants Thr394Thr and Gly482Ser of peroxisome proliferator-activated receptor gamma coactivator 1- $\alpha$  were also significantly associated with efficacy of rosiglitazone.

#### Adipocytokines

Variants of adiponectin (*ADIPOQ*) gene have been reported for changes in FPG and level of HbA1c after 12 wk of rosiglitazone treatment. A study carried out by Liu *et al.*<sup>[127]</sup> in T2DM Chinese patients demonstrated that sequence variation in leptin and TNF  $\alpha$  gene interferes with therapeutic response to rosiglitazone. Nucleotide variants rs2241766 (45T/G) and rs266729 (-11377C/G) of *ADIPOQ* gene<sup>[128]</sup>, rs1800629 (-308 G/A) of TNF- $\alpha$  and rs7799039 (-2548G/A) of leptin gene<sup>[127]</sup> were found to affect the rosiglitazone therapeutics and reverse insulin resistance in Chinese patients. In a pilot study, it was found that single nucleotide polymorphism at -420 (G/G) in resistin gene may serve as an independent predictor for down regulation of insulin resistance and hyperglycemia associated with pioglitazone therapeutics<sup>[129]</sup>.

#### Cytochrome P450

Metabolism of rosiglitazone is mainly metabolized by



**Figure 4** Schematic representations of peroxisome proliferator-activated receptor  $\gamma$  variants affecting the efficacy of thiazolidinediones. TZDs: Thiazolidinediones; PPAR $\gamma$ : Peroxisome proliferator-activated receptor  $\gamma$ .

*CYP2C8* and *CYP2C9*<sup>[78]</sup> while biotransformation of pioglitazone is mainly metabolized by *CYP2C8* and *CYP3A4*<sup>[130]</sup>. Nucleotide polymorphisms in *CYP2C8* gene were significantly associated with impaired clearance of rosiglitazone. Polymorphisms in *CYP2C8*\*3 encoding for a reduced functioning of *CYP2C8* enzyme, was reported for altered drug clearance<sup>[131]</sup>. Hence genetic variants of *CYP2C8* may contribute to the degree of TZD therapeutics.

## MEGLITINIDE

Meglitinide, insulin secretagogues act by inhibiting KATP channel leading to promote insulin secretion. Molecular mechanism of both sulphonylureas and meglitinide are similar. Sulphonylureas and meglitinide inhibit the activity of KATP channel by binding at two different sites of the SUR1 subunit<sup>[132]</sup>. Meglitinides have shorter duration of action and more rapid onset as compared with SUs. Repaglinide (a benzoic acid derivative) and nateglinide (a derivative of d-phenylalanine) belonging to meglitinide stimulate early secretion of insulin. Due to their short action, a potential adverse effect of meglitinide is to induce hypoglycemia<sup>[133]</sup>. Repaglinide is 100% metabolized in liver and hence excreted mainly *via* bile. Genetic polymorphisms associated with response to meglitinide were mapped in *SLCO1B1*, *CYP2C8*, *CYP3A4*, *TCF7L2*, *SLC30A8*, *IGF2BP2*, *KCNJ11*, *KCNQ1*, *UCP2*, *NAMPT*, *MDR1*, *PAX4* and *NeuroD1*<sup>[78,134-139]</sup>. Out of these *SLCO1B1* is reported to facilitate the hepatic uptake of a drug repaglinide<sup>[140]</sup>.

## GENES ASSOCIATED WITH MEGLITINIDE TREATMENT

### Solute Carrier Organic anion transporter family member 1B1

Solute Carrier Organic anion transporter family member 1B1 (*SLCO1B1*) gene, mainly expressed in basolateral membrane (hepatocytes) encodes for organic anion-

transporting polypeptide 1B1 (OATP1B1). Genetic polymorphisms in *SLCO1B1* have been reported to exert significant influence on repaglinide pharmacokinetics with reduced exposure after administration of a single dose of repaglinide<sup>[141]</sup>. Genetic variant of *SLCOB1* gene (521T > C) markedly affected the pharmacokinetics of nateglinide<sup>[134]</sup>. Cellular uptake of various drugs is regulated by OATP1B1. Several studies have demonstrated the pivotal role of *SLCOB1* gene variants in pharmacokinetics of meglitinides<sup>[134,142-144]</sup>. Nateglinide is catabolized by *CYP2C9*. A study performed in Chinese male volunteers has demonstrated that genetic variants of *SLCOB1* (521T > C) and *CYP2C9* (*CYP2C9*\*3) could affect the nateglinide efficacy<sup>[139]</sup>.

## OTHER GENES

*CYP2C8* and *CYP3A4*, both are actively engaged in metabolism of repaglinide. Clinical studies demonstrate that individuals with *CYP2C8*\*3 variant have greater clearance of OADs as compared to wild-type genotype<sup>[78]</sup>. A Chinese population treated with repaglinide and genotyped for *KCNQ1* variants rs2237892 (C/T) and rs2237895 (C/A) were found to be associated with therapeutic efficacy of repaglinide<sup>[137]</sup>. Single nucleotide polymorphisms in *SLC30A8* viz. Arg325Trp (rs13266634) and Arg325Gln (rs16889462) have been reported to be significantly associated with T2DM development and repaglinide efficacy<sup>[135]</sup>. *KCNJ11* SNP rs5219 (Lys23Glu) has been found to be associated with poor regulation of fasting/postprandial glucose and HbA1c levels in T2DM patients with "GA" or "AA" genotype in contrast with "GG". T2DM patients having "TT" genotype of *TCF7L2* gene rs290487 (C/T) demonstrated better efficacy for repaglinide treatment with respect to triglyceride, LDL and fasting insulin as compared to patients with "CC" or "CT" genotype<sup>[107]</sup>.

## DIPEPTIDYL PEPTIDASE 4

DPP-4 is involved in the degradation of two incretin

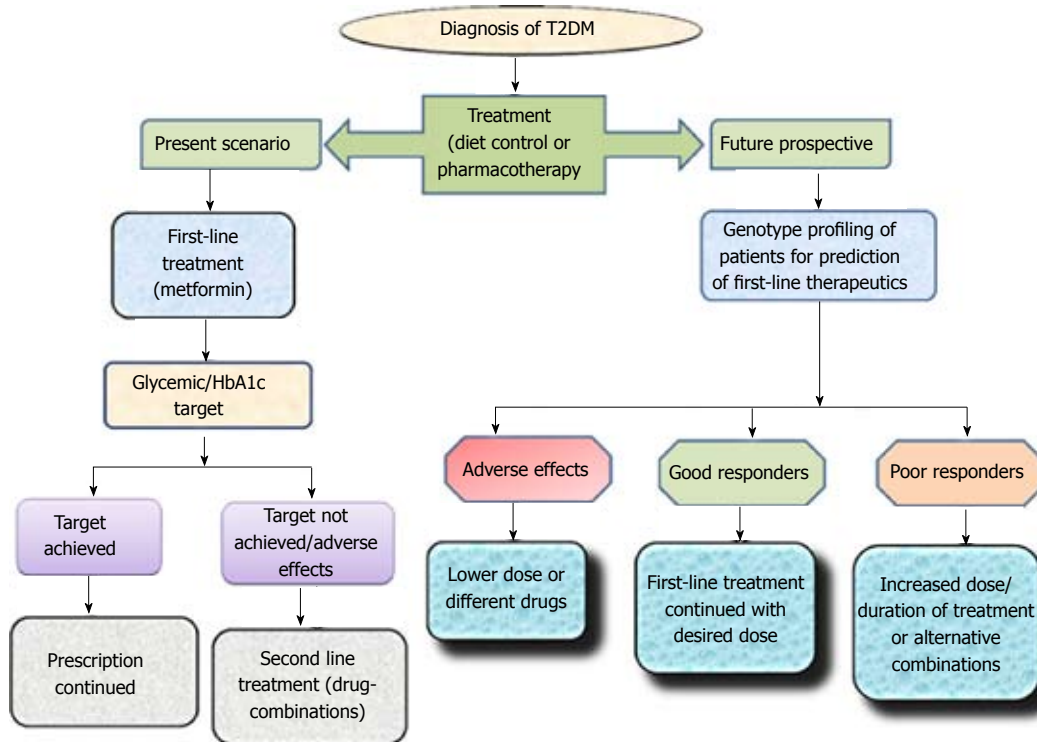


Figure 5 Clinical applications of pharmacogenetics in type 2 diabetes mellitus. T2DM: Type 2 diabetes mellitus; HbA1c: Glycated haemoglobin.

Table 1 Currently available antidiabetic drugs and their associated candidate genes involved in efficacy/toxicity

Class	Common medical representatives	Mechanism of action	Candidate genes involved in pharmacotherapy	Ref.
Biguanide	Metformin	AMP-kinase activation	<i>SLC22A1, SLC22A2, SLC22A3, SLC47A1, SLC47A2</i>	[28-39]
Sulfonylureas	Gliburide, gliclazide, Glimepiride, glipizide	Inhibition of KATP channel on plasma membrane of $\beta$ -cells	<i>KCNJ11, ABCC8, CYP2C9, TCF7L2</i>	[8,10,48-91]
Thiazolidinediones	Pioglitazone, rosiglitazone	Activates PPAR- $\gamma$	<i>PPAR-<math>\gamma</math>, ADIPOQ, TNF-<math>\alpha</math>, LEP, CYP2C8</i>	[92-131]
Meglitinides	Nateglinide, repaglinide	Inhibition of KATP channel on Plasma Membrane of $\beta$ -cells	<i>SLCOB1, CYP2C8, KCNQ1, SLC30A8, KCNJ11, TCF7L2</i>	[78,106,132-144]
DPP-4 inhibitors	Alogliptin, linagliptin, saxagliptin, sitagliptin, vildagliptin	Inhibits DPP-4, Affect GLP-1 receptor pathway	Possibly <i>TCF7L2</i>	[145-148]
$\alpha$ -glucosidase inhibitors	Acarbose, miglitol, voglibose	Inhibits intestinal $\alpha$ -glucosidase	Yet to identify?	[10]
SGLT-2 inhibitors	Canagliflozin, dapagliflozin, empagliflozin	Inhibits SGLT2 transporters in kidney	Yet to identify?	[10]
GLP-1 agonist	Exenatide, liraglutide	Activate GLP-1 receptor	Yet to identify?	[10]

DPP-4: Dipeptidyl peptidase-4; SGLT-2: Sodium glucose transporter-2; GLP-1: Glucagon like peptide-1; KATP: ATP-sensitive potassium channel; PPAR $\gamma$ : Peroxisome proliferator-activated receptor  $\gamma$ .

hormones viz. GLP-1 and gastric inhibitory polypeptide. These hormones bring about a glucose dependent stimulation of insulin release. These hormones are also responsible for reduction in circulating plasma glucose levels by interrupting glucagon secretion and subsequently improve beta cell sensitization by glucose<sup>[145]</sup>. DPP-4 inhibitors inhibit function of DPP-4 enzyme, thus reducing glucagon secretion. Sitagliptin, vildagliptin and saxagliptin are medical representatives of DPP4 inhibitors. Sitagliptin was the first DPP-4 inhibitor

approved by Food and Drug Administration (FDA) in 2006<sup>[146]</sup>. Metabolism of saxagliptin (a DPP-4 inhibitor) is catalyzed by CYP3A4/A5 while sitagliptin is metabolized by CYP3A4 with minor contribution of CYP2C8<sup>[147]</sup>. Zimdahl *et al.*<sup>[148]</sup> investigated the effect of *TCF7L2* variants for therapeutic efficacy of linagliptin, a DPP-4 inhibitor. Linagliptin was found to significantly improve glucose homeostasis in both cases with and without *TCF7L2* risk alleles for diabetes. Effects of genetic polymorphisms associated with DPP-4 inhibitors remain



to be investigated.

## CONCLUSION

About 70 genetic loci have been identified to be associated with T2DM<sup>[149]</sup>. Pharmacogenetics, an expanding area of research provides a platform to understand and improve pharmacological treatment. Over the last decade, the number of available antidiabetic drugs has considerably increased. However, clinical treatment of T2DM patients has become more complex due to different degrees of therapeutic outcomes. Personalized differences during OADs therapeutics have been linked with numerous variants related to drug-transporters, drug-targets, drug catabolizing enzymes and T2DM risk genes (Table 1). Although inter-individual differences in respect to efficacy and toxicity of OADs are significantly associated with genetic makeup, it is clear that different degrees of response to antidiabetics cannot be predicted by studying the genetic differences alone. The role of genetic variations with respect to therapeutic outcomes must be further tested *via* clinical trials thus leading to a personalized pharmacotherapy. The present scenario and future prospect of Pharmacogenetic studies has been elaborated in Figure 5.

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## REFERENCES

- 1 **IDF.** Diabetes Atlas, 7th ed. Brussels, Belgium: International Diabetes Federation, 2015: 1-114
- 2 **Saxena M,** Banerjee M. Diabetes: History, prevalence, insulin action and associated genes. *J Applied Bioscience* 2008; **34**: 139-151
- 3 **Saxena M,** Srivastava N, Banerjee M. Genetic association of adiponectin gene polymorphisms (+45T/G and +10211T/G) with type 2 diabetes in North Indians. *Diabetes Metab Syndr* 2012; **6**: 65-69 [PMID: 23153972 DOI: 10.1016/j.dsx.2012.08.008]
- 4 **Gautam S,** Pirabu L, Agrawal CG, Banerjee M. CD36 gene variants and their association with type 2 diabetes in an Indian population. *Diabetes Technol Ther* 2013; **15**: 680-687 [PMID: 23844572 DOI: 10.1089/dia.2012.0326]
- 5 **Saxena M,** Srivastava N, Banerjee M. Association of IL-6, TNF- $\alpha$  and IL-10 gene polymorphisms with type 2 diabetes mellitus. *Mol Biol Rep* 2013; **40**: 6271-6279 [PMID: 24057184 DOI: 10.1007/s11033-013-2739-4]
- 6 **Vats P,** Chandra H, Banerjee M. Glutathione S-transferase and catalase gene polymorphism with type 2 diabetes mellitus. *Diseases Mol Med* 2013; **1**: 46-53 [DOI: 10.5455/dmm.20131027101207]
- 7 **Vats P,** Sagar N, Singh TP, Banerjee M. Association of Superoxide dismutases (SOD1 and SOD2) and Glutathione peroxidase 1 (GPx1) gene polymorphisms with type 2 diabetes mellitus. *Free Radic Res* 2015; **49**: 17-24 [PMID: 25283363 DOI: 10.3109/10715762.2014.971782]
- 8 **Inzucchi SE,** Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, Peters AL, Tsapas A, Wender R, Matthews DR. Management of hyperglycemia in type 2 diabetes: a patient-centered approach: position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care* 2012; **35**: 1364-1379 [PMID: 22517736 DOI: 10.2337/dc12-0413]
- 9 **Topić E.** The Role of Pharmacogenetics in the Treatment of Diabetes Mellitus/uloga farmakogenetike u lečnju dijabetes melitusa. *J Med Biochem* 2014; **33**: 58-70 [DOI: 10.2478/jomb-2013-0045]
- 10 **American Diabetes Association.** Standards of medical care in diabetes--2006. *Diabetes Care* 2006; **29** Suppl 1: S4-42 [PMID: 16373931 DOI: 10.2337/dc16-S010]
- 11 **Mannino GC,** Sesti G. Individualized therapy for type 2 diabetes: clinical implications of pharmacogenetic data. *Mol Diagn Ther* 2012; **16**: 285-302 [PMID: 23018631]
- 12 **Vogel F.** Moderne probleme der Humangenetik. *Ergeb Inn Med Kinderheild* 1959; **12**: 52-125 [DOI: 10.1007/978-3-642-94744-5\_2]
- 13 **Hughes HB,** Biehl JP, Jones AP, Schmidt LH. Metabolism of isoniazid in man as related to the occurrence of peripheral neuritis. *Am Rev Tuberc* 1954; **70**: 266-273 [PMID: 13180863]
- 14 **Alving AS,** Carson PE, Flanagan CL, Ickes CE. Enzymatic deficiency in primaquine-sensitive erythrocytes. *Science* 1956; **124**: 484-485 [PMID: 13360274 DOI: 10.1126/science.124.3220.484-a]
- 15 **Evans DA,** Manley KA, Mckusick VA. Genetic control of isoniazid metabolism in man. *Br Med J* 1960; **2**: 485-491 [PMID: 13820968 DOI: 10.1136/bmj.2.5197.485]
- 16 **Evans WE,** Relling MV. Pharmacogenomics: translating functional genomics into rational therapeutics. *Science* 1999; **286**: 487-491 [PMID: 10521338 DOI: 10.1126/science.286.5439.487]
- 17 **Evans WE,** Johnson JA. Pharmacogenomics: the inherited basis for interindividual differences in drug response. *Annu Rev Genomics Hum Genet* 2001; **2**: 9-39 [PMID: 11701642 DOI: 10.1146/annurev.genom.2.1.9]
- 18 **McLeod HL,** Evans WE. Pharmacogenomics: unlocking the human genome for better drug therapy. *Annu Rev Pharmacol Toxicol* 2001; **41**: 101-121 [PMID: 11264452 DOI: 10.1146/annurev.pharmtox.41.1.101]
- 19 **Rettie AE,** Jones JP. Clinical and toxicological relevance of CYP2C9: drug-drug interactions and pharmacogenetics. *Annu Rev Pharmacol Toxicol* 2005; **45**: 477-494 [PMID: 15822186 DOI: 10.1146/annurev.pharmtox.45.120403.095821]
- 20 **American Diabetes Association.** Standards of medical care in diabetes--2013. *Diabetes Care* 2013; **36** Suppl 1: S11-S66 [PMID: 23264422 DOI: 10.2337/dc13-S011]
- 21 **American Diabetes Association.** Standards of medical care in diabetes--2012. *Diabetes Care* 2012; **35** Suppl 1: S11-S63 [PMID: 22187469 DOI: 10.2337/dc12-S011]
- 22 **Kirpichnikov D,** McFarlane SI, Sowers JR. Metformin: an update. *Ann Intern Med* 2002; **137**: 25-33 [PMID: 12093242 DOI: 10.7326/0003-4819-137-1-200207020-00009]
- 23 **Owen MR,** Doran E, Halestrap AP. Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. *Biochem J* 2000; **348** Pt 3: 607-614 [PMID: 10839993]
- 24 **Zhou G,** Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, Wu M, Ventre J, Doebber T, Fujii N, Musi N, Hirshman MF, Goodyear LJ, Moller DE. Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest* 2001; **108**: 1167-1174 [PMID: 11602624 DOI: 10.1172/JCI13505]
- 25 **Viollet B,** Guigas B, Sanz Garcia N, Leclerc J, Foretz M, Andreelli F. Cellular and molecular mechanisms of metformin: an overview. *Clin Sci (Lond)* 2012; **122**: 253-270 [PMID: 22117616 DOI: 10.1042/CS20110386]
- 26 **Otsuka M,** Matsumoto T, Morimoto R, Arioka S, Omote H, Moriyama Y. A human transporter protein that mediates the final excretion step for toxic organic cations. *Proc Natl Acad Sci USA* 2005; **102**: 17923-17928 [PMID: 16330770 DOI: 10.1073/pnas.0506483102]
- 27 **Masuda S,** Terada T, Yonezawa A, Tanihara Y, Kishimoto K, Katsura T, Ogawa O, Inui K. Identification and functional charact-

- erization of a new human kidney-specific H<sup>+</sup>/organic cation antiporter, kidney-specific multidrug and toxin extrusion 2. *J Am Soc Nephrol* 2006; **17**: 2127-2135 [PMID: 16807400 DOI: 10.1681/ASN.2006030205]
- 28 **Jacobs C**, Pearce B, Du Plessis M, Hoosain N, Benjeddou M. Genetic polymorphisms and haplotypes of the organic cation transporter 1 gene (SLC22A1) in the Xhosa population of South Africa. *Genet Mol Biol* 2014; **37**: 350-359 [PMID: 25071399 DOI: 10.1590/S1415-47572014005000002]
  - 29 **Umamaheswaran G**, Praveen RG, Damodaran SE, Das AK, Adithan C. Influence of SLC22A1 rs622342 genetic polymorphism on metformin response in South Indian type 2 diabetes mellitus patients. *Clin Exp Med* 2015; **15**: 511-517 [PMID: 25492374 DOI: 10.1007/s10238-014-0322-5]
  - 30 **Xiao D**, Guo Y, Li X, Yin JY, Zheng W, Qiu XW, Xiao L, Liu RR, Wang SY, Gong WJ, Zhou HH, Liu ZQ. The Impacts of SLC22A1 rs594709 and SLC47A1 rs2289669 Polymorphisms on Metformin Therapeutic Efficacy in Chinese Type 2 Diabetes Patients. *Int J Endocrinol* 2016; **2016**: 4350712 [PMID: 26977146 DOI: 10.1155/2016/4350712]
  - 31 **Shu Y**, Brown C, Castro RA, Shi RJ, Lin ET, Owen RP, Sheardown SA, Yue L, Burchard EG, Brett CM, Giacomini KM. Effect of genetic variation in the organic cation transporter 1, OCT1, on metformin pharmacokinetics. *Clin Pharmacol Ther* 2008; **83**: 273-280 [PMID: 17609683 DOI: 10.1038/sj.clpt.6100275]
  - 32 **Zhou K**, Donnelly LA, Kimber CH, Donnan PT, Doney AS, Leese G, Hattersley AT, McCarthy MI, Morris AD, Palmer CN, Pearson ER. Reduced-function SLC22A1 polymorphisms encoding organic cation transporter 1 and glycemic response to metformin: a GoDARTS study. *Diabetes* 2009; **58**: 1434-1439 [PMID: 19336679 DOI: 10.2337/db08-0896]
  - 33 **Motohashi H**, Sakurai Y, Saito H, Masuda S, Urakami Y, Goto M, Fukatsu A, Ogawa O, Inui K. Gene expression levels and immunolocalization of organic ion transporters in the human kidney. *J Am Soc Nephrol* 2002; **13**: 866-874 [PMID: 11912245]
  - 34 **Wang ZJ**, Yin OQ, Tomlinson B, Chow MS. OCT2 polymorphisms and in-vivo renal functional consequence: studies with metformin and cimetidine. *Pharmacogenet Genomics* 2008; **18**: 637-645 [PMID: 18551044 DOI: 10.1097/FPC.0b013e328302cd41]
  - 35 **Leabman MK**, Huang CC, Kawamoto M, Johns SJ, Stryke D, Ferrin TE, DeYoung J, Taylor T, Clark AG, Herskowitz I, Giacomini KM. Polymorphisms in a human kidney xenobiotic transporter, OCT2, exhibit altered function. *Pharmacogenetics* 2002; **12**: 395-405 [PMID: 12142729 DOI: 10.1097/00008571-200207000-00007]
  - 36 **Ogasawara K**, Terada T, Motohashi H, Asaka J, Aoki M, Katsura T, Kamba T, Ogawa O, Inui K. Analysis of regulatory polymorphisms in organic ion transporter genes (SLC22A) in the kidney. *J Hum Genet* 2008; **53**: 607-614 [PMID: 18414781 DOI: 10.1007/s10038-008-0288-9]
  - 37 **Takane H**, Shikata E, Otsubo K, Higuchi S, Ieiri I. Polymorphism in human organic cation transporters and metformin action. *Pharmacogenomics* 2008; **9**: 415-422 [PMID: 18384255 DOI: 10.2217/14622416.9.4.415]
  - 38 **Zolk O**, Solbach TF, König J, Fromm MF. Functional characterization of the human organic cation transporter 2 variant p.270Ala>gt; Ser. *Drug Metab Dispos* 2009; **37**: 1312-1318 [PMID: 19251820 DOI: 10.1124/dmd.108.023762]
  - 39 **Chen Y**, Li S, Brown C, Cheatham S, Castro RA, Leabman MK, Urban TJ, Chen L, Yee SW, Choi JH, Huang Y, Brett CM, Burchard EG, Giacomini KM. Effect of genetic variation in the organic cation transporter 2 on the renal elimination of metformin. *Pharmacogenet Genomics* 2009; **19**: 497-504 [PMID: 19483665 DOI: 10.1097/FPC.0b013e32832cc7e9]
  - 40 **Song IS**, Shin HJ, Shim EJ, Jung IS, Kim WY, Shon JH, Shin JG. Genetic variants of the organic cation transporter 2 influence the disposition of metformin. *Clin Pharmacol Ther* 2008; **84**: 559-562 [PMID: 18401339 DOI: 10.1038/clpt.2008.61]
  - 41 **Hou W**, Zhang D, Lu W, Zheng T, Wan L, Li Q, Bao Y, Liu F, Jia W. Polymorphism of organic cation transporter 2 improves glucose-lowering effect of metformin via influencing its pharmacokinetics in Chinese type 2 diabetic patients. *Mol Diagn Ther* 2015; **19**: 25-33 [PMID: 25573751 DOI: 10.1007/s40291-014-0126-z]
  - 42 **Sakata T**, Anzai N, Kimura T, Miura D, Fukutomi T, Takeda M, Sakurai H, Endou H. Functional analysis of human organic cation transporter OCT3 (SLC22A3) polymorphisms. *J Pharmacol Sci* 2010; **113**: 263-266 [PMID: 20562519 DOI: 10.1254/jphs.09331SC]
  - 43 **Chen L**, Pawlikowski B, Schlessinger A, More SS, Stryke D, Johns SJ, Portman MA, Chen E, Ferrin TE, Sali A, Giacomini KM. Role of organic cation transporter 3 (SLC22A3) and its missense variants in the pharmacologic action of metformin. *Pharmacogenet Genomics* 2010; **20**: 687-699 [PMID: 20859243 DOI: 10.1097/FPC.0b013e32833fe789]
  - 44 **Becker ML**, Visser LE, van Schaik RH, Hofman A, Uitterlinden AG, Stricker BH. Genetic variation in the multidrug and toxin extrusion 1 transporter protein influences the glucose-lowering effect of metformin in patients with diabetes: a preliminary study. *Diabetes* 2009; **58**: 745-749 [PMID: 19228809 DOI: 10.2337/db08-1028]
  - 45 **Jablonski KA**, McAteer JB, de Bakker PI, Franks PW, Pollin TI, Hanson RL, Saxena R, Fowler S, Shuldiner AR, Knowler WC, Altshuler D, Florez JC. Common variants in 40 genes assessed for diabetes incidence and response to metformin and lifestyle intervention in the diabetes prevention program. *Diabetes* 2010; **59**: 2672-2681 [PMID: 20682687 DOI: 10.2337/db10-0543]
  - 46 **He R**, Zhang D, Lu W, Zheng T, Wan L, Liu F, Jia W. SLC47A1 gene rs2289669 G>A; A variants enhance the glucose-lowering effect of metformin via delaying its excretion in Chinese type 2 diabetes patients. *Diabetes Res Clin Pract* 2015; **109**: 57-63 [PMID: 26004431 DOI: 10.1016/j.diabetes.2015.05.003]
  - 47 **Choi JH**, Yee SW, Ramirez AH, Morrissey KM, Jang GH, Joski PJ, Mefford JA, Hesselton SE, Schlessinger A, Jenkins G, Castro RA, Johns SJ, Stryke D, Sali A, Ferrin TE, Witte JS, Kwok PY, Roden DM, Wilke RA, McCarty CA, Davis RL, Giacomini KM. A common 5'-UTR variant in MATE2-K is associated with poor response to metformin. *Clin Pharmacol Ther* 2011; **90**: 674-684 [PMID: 21956618 DOI: 10.1038/clpt.2011.165]
  - 48 **Sola D**, Rossi L, Schianca GP, Maffioli P, Bigliocca M, Mella R, Corliano F, Fra GP, Bartoli E, Derosa G. Sulfonylureas and their use in clinical practice. *Arch Med Sci* 2015; **11**: 840-848 [PMID: 26322096 DOI: 10.5114/aoms.2015.53304]
  - 49 **Ashcroft FM**, Rorsman P. Electrophysiology of the pancreatic beta-cell. *Prog Biophys Mol Biol* 1989; **54**: 87-143 [PMID: 2484976 DOI: 10.1016/0079-6107(89)90013-8]
  - 50 **Flanagan SE**, Clauin S, Bellanné-Chantelot C, de Lonlay P, Harries LW, Gloyn AL, Ellard S. Update of mutations in the genes encoding the pancreatic beta-cell K(ATP) channel subunits Kir6.2 (KCNJ11) and sulfonylurea receptor 1 (ABCC8) in diabetes mellitus and hyperinsulinism. *Hum Mutat* 2009; **30**: 170-180 [PMID: 18767144 DOI: 10.1002/humu.20838]
  - 51 **Bozkurt O**, de Boer A, Grobbee DE, Heerdink ER, Burger H, Klungel OH. Pharmacogenetics of glucose-lowering drug treatment: a systematic review. *Mol Diagn Ther* 2007; **11**: 291-302 [PMID: 17963417 DOI: 10.1007/BF03256250]
  - 52 **Xu H**, Murray M, McLachlan AJ. Influence of genetic polymorphisms on the pharmacokinetics and pharmacodynamics of sulfonylurea drugs. *Curr Drug Metab* 2009; **10**: 643-658 [PMID: 19799532]
  - 53 **Bonfanti DH**, Alcazar LP, Arakaki PA, Martins LT, Agustini BC, de Moraes Rego FG, Frigeri HR. ATP-dependent potassium channels and type 2 diabetes mellitus. *Clin Biochem* 2015; **48**: 476-482 [PMID: 25583094 DOI: 10.1016/j.clinbiochem.2014.12.026]
  - 54 **Haghvirdizadeh P**, Mohamed Z, Abdullah NA, Haghvirdizadeh P, Haerian MS, Haerian BS. KCNJ11: Genetic Polymorphisms and Risk of Diabetes Mellitus. *J Diabetes Res* 2015; **2015**: 908152 [PMID: 26448950 DOI: 10.1155/2015/908152]
  - 55 **Zhou D**, Zhang D, Liu Y, Zhao T, Chen Z, Liu Z, Yu L, Zhang Z, Xu H, He L. The E23K variation in the KCNJ11 gene is associated with type 2 diabetes in Chinese and East Asian population. *J*

- Hum Genet* 2009; **54**: 433-435 [PMID: 19498446 DOI: 10.1038/jhg.2009.54]
- 56 **Phani NM**, Guddattu V, Bellampalli R, Seenappa V, Adhikari P, Nagri SK, D Souza SC, Mundyat GP, Satyamoorthy K, Rai PS. Population specific impact of genetic variants in KCNJ11 gene to type 2 diabetes: a case-control and meta-analysis study. *PLoS One* 2014; **9**: e107021 [PMID: 25247988 DOI: 10.1371/journal.pone.0107021]
  - 57 **Nikolac N**, Simundic AM, Katalinic D, Topic E, Cipak A, Zjadic Rotkovic V. Metabolic control in type 2 diabetes is associated with sulfonylurea receptor-1 (SUR-1) but not with KCNJ11 polymorphisms. *Arch Med Res* 2009; **40**: 387-392 [PMID: 19766903 DOI: 10.1016/j.arcmed.2009.06.006]
  - 58 **Klen J**, Dolzan V, Janež A. CYP2C9, KCNJ11 and ABCC8 polymorphisms and the response to sulphonylurea treatment in type 2 diabetes patients. *Eur J Clin Pharmacol* 2014; **70**: 421-428 [PMID: 24442125 DOI: 10.1007/s00228-014-1641-x]
  - 59 **Pearson ER**, Flechtner I, Njølstad PR, Malecki MT, Flanagan SE, Larkin B, Ashcroft FM, Klimes I, Codner E, Iotova V, Slingerland AS, Shield J, Robert JJ, Holst JJ, Clark PM, Ellard S, Søvik O, Polak M, Hattersley AT. Switching from insulin to oral sulfonylureas in patients with diabetes due to Kir6.2 mutations. *N Engl J Med* 2006; **355**: 467-477 [PMID: 16885550 DOI: 10.1056/NEJMOA061759]
  - 60 **Siklar Z**, Ellard S, Okulu E, Berberoğlu M, Young E, Savaş Erdevi S, Mungan IA, Hacıhamdioğlu B, Erdevi O, Arsan S, Oçal G. Transient neonatal diabetes with two novel mutations in the KCNJ11 gene and response to sulfonylurea treatment in a preterm infant. *J Pediatr Endocrinol Metab* 2011; **24**: 1077-1080 [PMID: 22308870 DOI: 10.1515/JPEM.2011.250]
  - 61 **Dupont J**, Pereira C, Medeira A, Duarte R, Ellard S, Sampaio L. Permanent neonatal diabetes mellitus due to KCNJ11 mutation in a Portuguese family: transition from insulin to oral sulfonylureas. *J Pediatr Endocrinol Metab* 2012; **25**: 367-370 [PMID: 22768671 DOI: 10.1515/jpem-2011-0191]
  - 62 **Patch AM**, Flanagan SE, Boustred C, Hattersley AT, Ellard S. Mutations in the ABCC8 gene encoding the SUR1 subunit of the KATP channel cause transient neonatal diabetes, permanent neonatal diabetes or permanent diabetes diagnosed outside the neonatal period. *Diabetes Obes Metab* 2007; **9** Suppl 2: 28-39 [PMID: 17919176 DOI: 10.1111/j.1463-1326.2007.00772.x]
  - 63 **Inoue H**, Ferrer J, Welling CM, Elbein SC, Hoffman M, Mayorga R, Warren-Perry M, Zhang Y, Millins H, Turner R, Province M, Bryan J, Permutt MA, Aguilar-Bryan L. Sequence variants in the sulfonylurea receptor (SUR) gene are associated with NIDDM in Caucasians. *Diabetes* 1996; **45**: 825-831 [PMID: 8635661 DOI: 10.2337/diab.45.6.825]
  - 64 **Hani EH**, Clément K, Velho G, Vionnet N, Hager J, Philippi A, Dina C, Inoue H, Permutt MA, Basdevant A, North M, Demeinai F, Guy-Grand B, Froguel P. Genetic studies of the sulfonylurea receptor gene locus in NIDDM and in morbid obesity among French Caucasians. *Diabetes* 1997; **46**: 688-694 [PMID: 9075812 DOI: 10.2337/diab.46.4.688]
  - 65 **Hart LM**, de Knijff P, Dekker JM, Stolk RP, Nijpels G, van der Does FE, Ruige JB, Grobbee DE, Heine RJ, Maassen JA. Variants in the sulphonylurea receptor gene: association of the exon 16-3t variant with Type II diabetes mellitus in Dutch Caucasians. *Diabetologia* 1999; **42**: 617-620 [PMID: 10333056 DOI: 10.1007/s001250051203]
  - 66 **Hart LM**, Dekker JM, van Haeften TW, Ruige JB, Stehouwer CD, Erkelens DW, Heine RJ, Maassen JA. Reduced second phase insulin secretion in carriers of a sulphonylurea receptor gene variant associating with Type II diabetes mellitus. *Diabetologia* 2000; **43**: 515-519 [PMID: 10819247 DOI: 10.1007/s001250051337]
  - 67 **Rissanen J**, Markkanen A, Kärkkäinen P, Pihlajamäki J, Kekäläinen P, Mykkänen L, Kuusisto J, Karhapää P, Niskanen L, Laakso M. Sulfonylurea receptor 1 gene variants are associated with gestational diabetes and type 2 diabetes but not with altered secretion of insulin. *Diabetes Care* 2000; **23**: 70-73 [PMID: 10857971 DOI: 10.2337/diacare.23.1.70]
  - 68 **Weisnagel SJ**, Rankinen T, Nadeau A, Rao DC, Chagnon YC, Pérusse L, Bouchard C. Decreased fasting and oral glucose stimulated C-peptide in nondiabetic subjects with sequence variants in the sulfonylurea receptor 1 gene. *Diabetes* 2001; **50**: 697-702 [PMID: 11246895 DOI: 10.2337/diabetes.50.3.697]
  - 69 **Reis AF**, Hani EH, Beressi N, Robert JJ, Bresson JL, Froguel P, Velho G. Allelic variation in exon 18 of the sulfonylurea receptor 1 (SUR1) gene, insulin secretion and insulin sensitivity in nondiabetic relatives of type 2 diabetic subjects. *Diabetes Metab* 2002; **28**: 209-215 [PMID: 12149601]
  - 70 **Venkatesan R**, Bodhini D, Narayani N, Mohan V. Association study of the ABCC8 gene variants with type 2 diabetes in south Indians. *Indian J Hum Genet* 2014; **20**: 37-42 [PMID: 24959012 DOI: 10.4103/0971-6866.132752]
  - 71 **Zhang H**, Liu X, Kuang H, Yi R, Xing H. Association of sulfonylurea receptor 1 genotype with therapeutic response to gliclazide in type 2 diabetes. *Diabetes Res Clin Pract* 2007; **77**: 58-61 [PMID: 17118480 DOI: 10.1016/j.diabres.2006.10.021]
  - 72 **Sesti G**, Marini MA, Cardellini M, Sciacqua A, Frontoni S, Andreozzi F, Irace C, Lauro D, Gnasso A, Federici M, Perticone F, Lauro R. The Arg972 variant in insulin receptor substrate-1 is associated with an increased risk of secondary failure to sulfonylurea in patients with type 2 diabetes. *Diabetes Care* 2004; **27**: 1394-1398 [PMID: 15161794 DOI: 10.2337/diacare.27.6.1394]
  - 73 **Feng Y**, Mao G, Ren X, Xing H, Tang G, Li Q, Li X, Sun L, Yang J, Ma W, Wang X, Xu X. Ser1369Ala variant in sulfonylurea receptor gene ABCC8 is associated with antidiabetic efficacy of gliclazide in Chinese type 2 diabetic patients. *Diabetes Care* 2008; **31**: 1939-1944 [PMID: 18599530 DOI: 10.2337/dc07-2248]
  - 74 **Hansen T**, Ambye L, Grarup N, Hansen L, Echwald SM, Ferrer J, Pedersen O. Genetic variability of the SUR1 promoter in relation to beta-cell function and Type II diabetes mellitus. *Diabetologia* 2001; **44**: 1330-1334 [PMID: 11692183 DOI: 10.1007/s001250100651]
  - 75 **Holstein A**, Plaschke A, Ptak M, Egberts EH, El-Din J, Brockmöller J, Kirchheiner J. Association between CYP2C9 slow metabolizer genotypes and severe hypoglycaemia on medication with sulphonylurea hypoglycaemic agents. *Br J Clin Pharmacol* 2005; **60**: 103-106 [PMID: 15963101]
  - 76 **Kirchheiner J**, Bauer S, Meineke I, Rohde W, Prang V, Meisel C, Roots I, Brockmöller J. Impact of CYP2C9 and CYP2C19 polymorphisms on tolbutamide kinetics and the insulin and glucose response in healthy volunteers. *Pharmacogenetics* 2002; **12**: 101-109 [PMID: 11875364 DOI: 10.1097/00008571-200203000-00004]
  - 77 **Becker ML**, Visser LE, Trienekens PH, Hofman A, van Schaik RH, Stricker BH. Cytochrome P450 2C9 \*2 and \*3 polymorphisms and the dose and effect of sulfonylurea in type II diabetes mellitus. *Clin Pharmacol Ther* 2008; **83**: 288-292 [PMID: 17597710 DOI: 10.1038/sj.clpt.6100273]
  - 78 **Kirchheiner J**, Roots I, Goldammer M, Rosenkranz B, Brockmöller J. Effect of genetic polymorphisms in cytochrome p450 (CYP) 2C9 and CYP2C8 on the pharmacokinetics of oral antidiabetic drugs: clinical relevance. *Clin Pharmacokinet* 2005; **44**: 1209-1225 [PMID: 16372821 DOI: 10.2165/00003088-200544120-00002]
  - 79 **Ragia G**, Petridis I, Tavridou A, Christakidis D, Manolopoulos VG. Presence of CYP2C9\*3 allele increases risk for hypoglycemia in Type 2 diabetic patients treated with sulfonylureas. *Pharmacogenomics* 2009; **10**: 1781-1787 [PMID: 19891554 DOI: 10.2217/Pgs.09.96]
  - 80 **Rydberg T**, Jönsson A, Roder M, Melander A. Hypoglycemic activity of glyburide (glibenclamide) metabolites in humans. *Diabetes Care* 1994; **17**: 1026-1030 [PMID: 7988301 DOI: 10.2337/diacare.17.9.1026]
  - 81 **Distefano JK**, Watanabe RM. Pharmacogenetics of Anti-Diabetes Drugs. *Pharmaceuticals* (Basel) 2010; **3**: 2610-2646 [PMID: 20936101 DOI: 10.3390/ph3082610]
  - 82 **Zhou Y**, Park SY, Su J, Bailey K, Ottosson-Laakso E, Shcherbina L, Oskolkov N, Zhang E, Thevenin T, Fadista J, Bennet H, Vikman P, Wierup N, Fex M, Rung J, Wollheim C, Nobrega M, Renström E, Groop L, Hansson O. TCF7L2 is a master regulator of insulin production and processing. *Hum Mol Genet* 2014; **23**: 6419-6431 [PMID: 25015099 DOI: 10.1093/hmg/ddu359]



- 83 **Cauchi S**, Meyre D, Dina C, Choquet H, Samson C, Gallina S, Balkau B, Charpentier G, Pattou F, Stetsyuk V, Scharfmann R, Staels B, Frühbeck G, Froguel P. Transcription factor TCF7L2 genetic study in the French population: expression in human beta-cells and adipose tissue and strong association with type 2 diabetes. *Diabetes* 2006; **55**: 2903-2908 [PMID: 17003360 DOI: 10.2337/db06-0474]
- 84 **Florez JC**, Jablonski KA, Bayley N, Pollin TI, de Bakker PI, Shuldiner AR, Knowler WC, Nathan DM, Altshuler D. TCF7L2 polymorphisms and progression to diabetes in the Diabetes Prevention Program. *N Engl J Med* 2006; **355**: 241-250 [PMID: 16855264 DOI: 10.1056/NEJMoa062418]
- 85 **Melzer D**, Murray A, Hurst AJ, Weedon MN, Bandinelli S, Corsi AM, Ferrucci L, Paolisso G, Guralnik JM, Frayling TM. Effects of the diabetes linked TCF7L2 polymorphism in a representative older population. *BMC Med* 2006; **4**: 34 [PMID: 17181866 DOI: 10.1186/1741-7015-4-34]
- 86 **Saxena R**, Gianniny L, Burt NP, Lyssenko V, Giuducci C, Sjögren M, Florez JC, Almgren P, Isomaa B, Orho-Melander M, Lindblad U, Daly MJ, Tuomi T, Hirschhorn JN, Ardlie KG, Groop LC, Altshuler D. Common single nucleotide polymorphisms in TCF7L2 are reproducibly associated with type 2 diabetes and reduce the insulin response to glucose in nondiabetic individuals. *Diabetes* 2006; **55**: 2890-2895 [PMID: 17003358 DOI: 10.2337/db06-0381]
- 87 **Miyake K**, Horikawa Y, Hara K, Yasuda K, Osawa H, Furuta H, Hirota Y, Yamagata K, Hinokio Y, Oka Y, Iwasaki N, Iwamoto Y, Yamada Y, Seino Y, Maegawa H, Kashiwagi A, Yamamoto K, Tokunaga K, Takeda J, Makino H, Nanjo K, Kadowaki T, Kasuga M. Association of TCF7L2 polymorphisms with susceptibility to type 2 diabetes in 4,087 Japanese subjects. *J Hum Genet* 2008; **53**: 174-180 [PMID: 18097733 DOI: 10.1007/s10038-007-0231-5]
- 88 **Pearson ER**, Donnelly LA, Kimber C, Whitley A, Doney AS, McCarthy MI, Hattersley AT, Morris AD, Palmer CN. Variation in TCF7L2 influences therapeutic response to sulfonylureas: a GoDARTs study. *Diabetes* 2007; **56**: 2178-2182 [PMID: 17519421 DOI: 10.2337/db07-0440]
- 89 **Brunetti A**, Chiefari E, Foti D. Recent advances in the molecular genetics of type 2 diabetes mellitus. *World J Diabetes* 2014; **5**: 128-140 [PMID: 24748926 DOI: 10.4239/wjd.v5.i2.128]
- 90 **Hattersley AT**. Prime suspect: the TCF7L2 gene and type 2 diabetes risk. *J Clin Invest* 2007; **117**: 2077-2079 [PMID: 17671643 DOI: 10.1172/JCI33077]
- 91 **Lyssenko V**, Lupi R, Marchetti P, Del Guerra S, Orho-Melander M, Almgren P, Sjögren M, Ling C, Eriksson KF, Lethagen AL, Mancarella R, Berglund G, Tuomi T, Nilsson P, Del Prato S, Groop L. Mechanisms by which common variants in the TCF7L2 gene increase risk of type 2 diabetes. *J Clin Invest* 2007; **117**: 2155-2163 [PMID: 17671651 DOI: 10.1172/JCI30706]
- 92 **Chen L**, Yang G. PPARs Integrate the Mammalian Clock and Energy Metabolism. *PPAR Res* 2014; **2014**: 653017 [PMID: 24693278 DOI: 10.1155/2014/653017]
- 93 **Spiegelman BM**. PPAR-gamma: adipogenic regulator and thiazolidinedione receptor. *Diabetes* 1998; **47**: 507-514 [PMID: 9568680 DOI: 10.2337/diabetes.47.4.507]
- 94 **Aronoff S**, Rosenblatt S, Braithwaite S, Egan JW, Mathisen AL, Schneider RL. Pioglitazone hydrochloride monotherapy improves glycaemic control in the treatment of patients with type 2 diabetes: a 6-month randomized placebo-controlled dose-response study. The Pioglitazone 001 Study Group. *Diabetes Care* 2000; **23**: 1605-1611 [PMID: 11092281 DOI: 10.2337/diacare.23.11.1605]
- 95 **Scherbaum WA**, Göke B. Metabolic efficacy and safety of once-daily pioglitazone monotherapy in patients with type 2 diabetes: a double-blind, placebo-controlled study. *Horm Metab Res* 2002; **34**: 589-595 [PMID: 12439788 DOI: 10.1055/s-2002-35421]
- 96 **Petersen KF**, Krssak M, Inzucchi S, Cline GW, Dufour S, Shulman GI. Mechanism of troglitazone action in type 2 diabetes. *Diabetes* 2000; **49**: 827-831 [PMID: 10905493 DOI: 10.2337/diabetes.49.5.827]
- 97 **Miyazaki Y**, Mahankali A, Matsuda M, Mahankali S, Hardies J, Cusi K, Mandarin LJ, DeFronzo RA. Effect of pioglitazone on abdominal fat distribution and insulin sensitivity in type 2 diabetic patients. *J Clin Endocrinol Metab* 2002; **87**: 2784-2791 [PMID: 12050251 DOI: 10.1210/jcem.87.6.8567]
- 98 **Bajaj M**, Suraamornkul S, Pratipanawatr T, Hardies LJ, Pratipanawatr W, Glass L, Cersosimo E, Miyazaki Y, DeFronzo RA. Pioglitazone reduces hepatic fat content and augments splanchnic glucose uptake in patients with type 2 diabetes. *Diabetes* 2003; **52**: 1364-1370 [PMID: 12765945 DOI: 10.2337/diabetes.52.6.1364]
- 99 **Buchanan TA**, Xiang AH, Peters RK, Kjos SL, Marroquin A, Goico J, Ochoa C, Tan S, Berkowitz K, Hodis HN, Azen SP. Preservation of pancreatic beta-cell function and prevention of type 2 diabetes by pharmacological treatment of insulin resistance in high-risk hispanic women. *Diabetes* 2002; **51**: 2796-2803 [PMID: 12196473 DOI: 10.2337/diabetes.51.9.2796]
- 100 **Ye JM**, Frangoudakis G, Iglesias MA, Furler SM, Ellis B, Dzamko N, Cooney GJ, Kraegen EW. Prior thiazolidinedione treatment preserves insulin sensitivity in normal rats during acute fatty acid elevation: role of the liver. *Endocrinology* 2002; **143**: 4527-4535 [PMID: 12446579 DOI: 10.1210/en.2002-220387]
- 101 **Maggs DG**, Buchanan TA, Burant CF, Cline G, Gumbiner B, Hsueh WA, Inzucchi S, Kelley D, Nolan J, Olefsky JM, Polonsky KS, Silver D, Valiquett TR, Shulman GI. Metabolic effects of troglitazone monotherapy in type 2 diabetes mellitus. A randomized, double-blind, placebo-controlled trial. *Ann Intern Med* 1998; **128**: 176-185 [PMID: 9454525 DOI: 10.7326/0003-4819-128-3-199802010-00002]
- 102 **Mayerson AB**, Hundal RS, Dufour S, Lebon V, Befroy D, Cline GW, Enoksson S, Inzucchi SE, Shulman GI, Petersen KF. The effects of rosiglitazone on insulin sensitivity, lipolysis, and hepatic and skeletal muscle triglyceride content in patients with type 2 diabetes. *Diabetes* 2002; **51**: 797-802 [PMID: 11872682 DOI: 10.2337/diabetes.51.3.797]
- 103 **Nakamura T**, Funahashi T, Yamashita S, Nishida M, Nishida Y, Takahashi M, Hotta K, Kuriyama H, Kihara S, Ohuchi N, Nishimura T, Kishino BI, Ishikawa K, Kawamoto T, Tokunaga K, Nakagawa C, Mineo I, Watanabe F, Tarui S, Matsuzawa Y. Thiazolidinedione derivative improves fat distribution and multiple risk factors in subjects with visceral fat accumulation--double-blind placebo-controlled trial. *Diabetes Res Clin Pract* 2001; **54**: 181-190 [PMID: 11689273 DOI: 10.1016/S0168-8227(01)00319-9]
- 104 **Sigrist S**, Bedoucha M, Boelsterli UA. Down-regulation by troglitazone of hepatic tumor necrosis factor-alpha and interleukin-6 mRNA expression in a murine model of non-insulin-dependent diabetes. *Biochem Pharmacol* 2000; **60**: 67-75 [PMID: 10807946 DOI: 10.1016/S0006-2952(00)00299-9]
- 105 **Alleva DG**, Johnson EB, Lio FM, Boehme SA, Conlon PJ, Crowe PD. Regulation of murine macrophage proinflammatory and anti-inflammatory cytokines by ligands for peroxisome proliferator-activated receptor-gamma: counter-regulatory activity by IFN-gamma. *J Leukoc Biol* 2002; **71**: 677-685 [PMID: 11927655]
- 106 **Albrektsson T**, Frederiksen KS, Holmes WE, Boel E, Taylor K, Fleckner J. Novel genes regulated by the insulin sensitizer rosiglitazone during adipocyte differentiation. *Diabetes* 2002; **51**: 1042-1051 [PMID: 11916924 DOI: 10.2337/diabetes.51.4.1042]
- 107 **Yu M**, Xu XJ, Yin JY, Wu J, Chen X, Gong ZC, Ren HY, Huang Q, Sheng FF, Zhou HH, Liu ZQ. KCNJ11 Lys23Glu and TCF7L2 rs290487(C/T) polymorphisms affect therapeutic efficacy of repaglinide in Chinese patients with type 2 diabetes. *Clin Pharmacol Ther* 2010; **87**: 330-335 [PMID: 20054294 DOI: 10.1038/clpt.2009.242]
- 108 **Iwaki M**, Matsuda M, Maeda N, Funahashi T, Matsuzawa Y, Makishima M, Shimomura I. Induction of adiponectin, a fat-derived antidiabetic and antiatherogenic factor, by nuclear receptors. *Diabetes* 2003; **52**: 1655-1663 [PMID: 12829629 DOI: 10.2337/diabetes.52.7.1655]
- 109 **Maeda N**, Shimomura I, Kishida K, Nishizawa H, Matsuda M, Nagaretani H, Furuyama N, Kondo H, Takahashi M, Arita Y, Komuro R, Ouchi N, Kihara S, Tochino Y, Okutomi K, Horie M, Takeda S, Aoyama T, Funahashi T, Matsuzawa Y. Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. *Nat Med* 2002; **8**: 731-737 [PMID: 12068289 DOI: 10.1038/nm724]
- 110 **Nissen SE**, Wolski K. Effect of rosiglitazone on the risk of



- myocardial infarction and death from cardiovascular causes. *N Engl J Med* 2007; **356**: 2457-2471 [PMID: 17517853 DOI: 10.1056/NEJMoa072761]
- 111 **Chen X**, Yang L, Zhai SD. Risk of cardiovascular disease and all-cause mortality among diabetic patients prescribed rosiglitazone or pioglitazone: a meta-analysis of retrospective cohort studies. *Chin Med J (Engl)* 2012; **125**: 4301-4306 [PMID: 23217404]
  - 112 **Semiz S**, Dujic T, Causevic A. Pharmacogenetics and personalized treatment of type 2 diabetes. *Biochem Med (Zagreb)* 2013; **23**: 154-171 [PMID: 23894862 DOI: 10.11613/BM.2013.020]
  - 113 **Mudaliar S**, Henry RR. New oral therapies for type 2 diabetes mellitus: The glitazones or insulin sensitizers. *Annu Rev Med* 2001; **52**: 239-257 [PMID: 11160777 DOI: 10.1146/annurev.med.52.1.239]
  - 114 **Rosen ED**, Sarraf P, Troy AE, Bradwin G, Moore K, Milstone DS, Spiegelman BM, Mortensen RM. PPAR gamma is required for the differentiation of adipose tissue in vivo and in vitro. *Mol Cell* 1999; **4**: 611-617 [PMID: 10549292 DOI: 10.1016/S1097-2765(00)80211-7]
  - 115 **Berger J**, Bailey P, Biswas C, Cullinan CA, Doebber TW, Hayes NS, Saperstein R, Smith RG, Leibowitz MD. Thiazolidinediones produce a conformational change in peroxisomal proliferator-activated receptor-gamma: binding and activation correlate with antidiabetic actions in db/db mice. *Endocrinology* 1996; **137**: 4189-4195 [PMID: 8828476 DOI: 10.1210/endo.137.10.8828476]
  - 116 **Schoonjans K**, Martin G, Staels B, Auwerx J. Peroxisome proliferator-activated receptors, orphans with ligands and functions. *Curr Opin Lipidol* 1997; **8**: 159-166 [PMID: 9211064 DOI: 10.1097/00041433-199706000-00006]
  - 117 **Hauner H**. The mode of action of thiazolidinediones. *Diabetes Metab Res Rev* 2002; **18** Suppl 2: S10-S15 [PMID: 11921433 DOI: 10.1002/dmrr.249]
  - 118 **Barroso I**, Gurnell M, Crowley VE, Agostini M, Schwabe JW, Soos MA, Maslen GL, Williams TD, Lewis H, Schafer AJ, Chatterjee VK, O'Rahilly S. Dominant negative mutations in human PPARgamma associated with severe insulin resistance, diabetes mellitus and hypertension. *Nature* 1999; **402**: 880-883 [PMID: 10622252]
  - 119 **Altshuler D**, Hirschhorn JN, Klannemark M, Lindgren CM, Vohl MC, Nemesh J, Lane CR, Schaffner SF, Bolk S, Brewer C, Tuomi T, Gaudet D, Hudson TJ, Daly M, Groop L, Lander ES. The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nat Genet* 2000; **26**: 76-80 [PMID: 10973253 DOI: 10.1038/79216]
  - 120 **Azab MM**, Abdel-Azeez HA, Zanaty MF, El Alawi SM. Peroxisome proliferator activated receptor  $\gamma$ 2 gene Pro12Ala gene polymorphism in type 2 diabetes and its relationship with diabetic nephropathy. *Clin Lab* 2014; **60**: 743-749 [PMID: 24839816]
  - 121 **Dubinina IA**, Chistiakov DA, Eremina IA, Brovkin AN, Zilberman LI, Nikitin AG, Kuraeva TL, Nosikov VV, Peterkova VA, Dedov II. Studying progression from glucose intolerance to type 2 diabetes in obese children. *Diabetes Metab Syndr* 2014; **8**: 133-137 [PMID: 25127329 DOI: 10.1016/j.dsx.2014.07.002]
  - 122 **Koch M**, Rett K, Maerker E, Volk A, Haist K, Deninger M, Renn W, Häring HU. The PPARgamma2 amino acid polymorphism Pro 12 Ala is prevalent in offspring of Type II diabetic patients and is associated to increased insulin sensitivity in a subgroup of obese subjects. *Diabetologia* 1999; **42**: 758-762 [PMID: 10382597 DOI: 10.1007/s001250051225]
  - 123 **Stumvoll M**, Wahl HG, Löblein K, Becker R, Machicao F, Jacob S, Häring H. Pro12Ala polymorphism in the peroxisome proliferator-activated receptor-gamma2 gene is associated with increased antilipolytic insulin sensitivity. *Diabetes* 2001; **50**: 876-881 [PMID: 11289055 DOI: 10.2337/diabetes.50]
  - 124 **Priya SS**, Sankaran R, Ramalingam S, Sairam T, Somasundaram LS. Genotype Phenotype Correlation of Genetic Polymorphism of PPAR Gamma Gene and Therapeutic Response to Pioglitazone in Type 2 Diabetes Mellitus- A Pilot Study. *J Clin Diagn Res* 2016; **10**: FC11-FC14 [PMID: 27042481 DOI: 10.7860/JCDR.2016/16494.7311]
  - 125 **Kang ES**, Park SY, Kim HJ, Kim CS, Ahn CW, Cha BS, Lim SK, Nam CM, Lee HC. Effects of Pro12Ala polymorphism of peroxisome proliferator-activated receptor gamma2 gene on rosiglitazone response in type 2 diabetes. *Clin Pharmacol Ther* 2005; **78**: 202-208 [PMID: 16084854 DOI: 10.1016/j.clpt.2005.04.013]
  - 126 **Zhang KH**, Huang Q, Dai XP, Yin JY, Zhang W, Zhou G, Zhou HH, Liu ZQ. Effects of the peroxisome proliferator activated receptor- $\gamma$  coactivator-1 $\alpha$  (PGC-1 $\alpha$ ) Thr394Thr and Gly482Ser polymorphisms on rosiglitazone response in Chinese patients with type 2 diabetes mellitus. *J Clin Pharmacol* 2010; **50**: 1022-1030 [PMID: 20498286 DOI: 10.1177/0091270009355159]
  - 127 **Liu HL**, Lin YG, Wu J, Sun H, Gong ZC, Hu PC, Yin JY, Zhang W, Wang D, Zhou HH, Liu ZQ. Impact of genetic polymorphisms of leptin and TNF-alpha on rosiglitazone response in Chinese patients with type 2 diabetes. *Eur J Clin Pharmacol* 2008; **64**: 663-671 [PMID: 18438653 DOI: 10.1007/s00228-008-0483-9]
  - 128 **Sun H**, Gong ZC, Yin JY, Liu HL, Liu YZ, Guo ZW, Zhou HH, Wu J, Liu ZQ. The association of adiponectin allele 45T/G and -11377C/G polymorphisms with Type 2 diabetes and rosiglitazone response in Chinese patients. *Br J Clin Pharmacol* 2008; **65**: 917-926 [PMID: 18429970 DOI: 10.1111/j.1365-2125.2008.03145.x]
  - 129 **Makino H**, Shimizu I, Murao S, Kondo S, Tabara Y, Fujiyama M, Fujii Y, Takada Y, Nakai K, Izumi K, Ohashi J, Kawamura R, Yamauchi J, Takata Y, Nishida W, Hashiramoto M, Onuma H, Osawa H. A pilot study suggests that the G/G genotype of resistin single nucleotide polymorphism at -420 may be an independent predictor of a reduction in fasting plasma glucose and insulin resistance by pioglitazone in type 2 diabetes. *Endocr J* 2009; **56**: 1049-1058 [PMID: 19738363 DOI: 10.1507/endocrj.K08E-320]
  - 130 **Jaakkola T**, Laitila J, Neuvonen PJ, Backman JT. Pioglitazone is metabolised by CYP2C8 and CYP3A4 in vitro: potential for interactions with CYP2C8 inhibitors. *Basic Clin Pharmacol Toxicol* 2006; **99**: 44-51 [PMID: 16867170 DOI: 10.1111/j.1742-7843.2006.pto\_437.x]
  - 131 **Kirchheiner J**, Thomas S, Bauer S, Tomalik-Scharte D, Hering U, Doroshenko O, Jetter A, Stehle S, Tshuridu M, Meineke I, Brockmüller J, Fuhr U. Pharmacokinetics and pharmacodynamics of rosiglitazone in relation to CYP2C8 genotype. *Clin Pharmacol Ther* 2006; **80**: 657-667 [PMID: 17178266 DOI: 10.1016/j.clpt.2006.09.008]
  - 132 **Yan FF**, Casey J, Shyng SL. Sulfonylureas correct trafficking defects of disease-causing ATP-sensitive potassium channels by binding to the channel complex. *J Biol Chem* 2006; **281**: 33403-33413 [PMID: 16956886 DOI: 10.1074/jbc.M605195200]
  - 133 **Huang C**, Florez JC. Pharmacogenetics in type 2 diabetes: potential implications for clinical practice. *Genome Med* 2011; **3**: 76 [PMID: 22126607 DOI: 10.1186/gm292]
  - 134 **Zhang W**, He YJ, Han CT, Liu ZQ, Li Q, Fan L, Tan ZR, Zhang WX, Yu BN, Wang D, Hu DL, Zhou HH. Effect of SLC01B1 genetic polymorphism on the pharmacokinetics of nateglinide. *Br J Clin Pharmacol* 2006; **62**: 567-572 [PMID: 16796707 DOI: 10.1111/j.1365-2125.2006.02686.x]
  - 135 **Huang Q**, Yin JY, Dai XP, Wu J, Chen X, Deng CS, Yu M, Gong ZC, Zhou HH, Liu ZQ. Association analysis of SLC30A8 rs13266634 and rs16889462 polymorphisms with type 2 diabetes mellitus and repaglinide response in Chinese patients. *Eur J Clin Pharmacol* 2010; **66**: 1207-1215 [PMID: 20809084 DOI: 10.1007/s00228-010-0882-6]
  - 136 **Sheng FF**, Dai XP, Qu J, Lei GH, Lu HB, Wu J, Xu XJ, Pei Q, Dong M, Liu YZ, Zhou HH, Liu ZQ. NAMPT -3186C/T polymorphism affects repaglinide response in Chinese patients with Type 2 diabetes mellitus. *Clin Exp Pharmacol Physiol* 2011; **38**: 550-554 [PMID: 21631570 DOI: 10.1111/j.1440-1681.2011.05548.x]
  - 137 **Dai XP**, Huang Q, Yin JY, Guo Y, Gong ZC, Lei MX, Jiang TJ, Zhou HH, Liu ZQ. KCNQ1 gene polymorphisms are associated with the therapeutic efficacy of repaglinide in Chinese type 2 diabetic patients. *Clin Exp Pharmacol Physiol* 2012; **39**: 462-468 [PMID: 22414228 DOI: 10.1111/j.1440-1681.2012.05701.x]
  - 138 **Xiang Q**, Cui YM, Zhao X, Yan L, Zhou Y. The Influence of MDR1 G2677T/a genetic polymorphisms on the pharmacokinetics of repaglinide in healthy Chinese volunteers. *Pharmacology* 2012; **89**: 105-110 [PMID: 22398664 DOI: 10.1159/000336345]
  - 139 **Cheng Y**, Wang G, Zhang W, Fan L, Chen Y, Zhou HH. Effect of

- CYP2C9 and SLCO1B1 polymorphisms on the pharmacokinetics and pharmacodynamics of nateglinide in healthy Chinese male volunteers. *Eur J Clin Pharmacol* 2013; **69**: 407-413 [PMID: 22842957 DOI: 10.1007/s00228-012-1364-9]
- 140 **Takane H.** Genetic polymorphisms of SLCO1B1 for drug pharmacokinetics and its clinical implications. *Yakugaku Zasshi* 2011; **131**: 1589-1594 [PMID: 22041697 DOI: 10.1248/yakushi.131.1589]
- 141 **He J, Qiu Z, Li N, Yu Y, Lu Y, Han D, Li T, Zhao D, Sun W, Fang F, Zheng J, Fan H, Chen X.** Effects of SLCO1B1 polymorphisms on the pharmacokinetics and pharmacodynamics of repaglinide in healthy Chinese volunteers. *Eur J Clin Pharmacol* 2011; **67**: 701-707 [PMID: 21327909 DOI: 10.1007/s00228-011-0994-7]
- 142 **Kalliokoski A, Backman JT, Neuvonen PJ, Niemi M.** Effects of the SLCO1B1\*1B haplotype on the pharmacokinetics and pharmacodynamics of repaglinide and nateglinide. *Pharmacogenet Genomics* 2008; **18**: 937-942 [PMID: 18854776 DOI: 10.1097/FPC.0B013E32830D733E]
- 143 **Kalliokoski A, Neuvonen M, Neuvonen PJ, Niemi M.** Different effects of SLCO1B1 polymorphism on the pharmacokinetics and pharmacodynamics of repaglinide and nateglinide. *J Clin Pharmacol* 2008; **48**: 311-321 [PMID: 18187595 DOI: 10.1177/0091270007311569]
- 144 **Kalliokoski A, Neuvonen M, Neuvonen PJ, Niemi M.** The effect of SLCO1B1 polymorphism on repaglinide pharmacokinetics persists over a wide dose range. *Br J Clin Pharmacol* 2008; **66**: 818-825 [PMID: 18823304 DOI: 10.1111/J.1365-2125.2008.03287.x]
- 145 **Holstein A, Seeringer A, Kovacs P.** Therapy with oral antidiabetic drugs: applied pharmacogenetics. *Br J Diabetes Vascular Disease* 2011; **11**: 10-16 [DOI: 10.1177/1474651410397583]
- 146 **Dicker D.** DPP-4 inhibitors: impact on glycemic control and cardiovascular risk factors. *Diabetes Care* 2011; **34** Suppl 2: S276-S278 [PMID: 21525468 DOI: 10.2337/dc11-s229]
- 147 **Vincent SH, Reed JR, Bergman AJ, Elmore CS, Zhu B, Xu S, Ebel D, Larson P, Zeng W, Chen L, Dilzer S, Lassetter K, Gottesdiener K, Wagner JA, Herman GA.** Metabolism and excretion of the dipeptidyl peptidase 4 inhibitor [14C]sitagliptin in humans. *Drug Metab Dispos* 2007; **35**: 533-538 [PMID: 17220239 DOI: 10.1124/dmd.106.013136]
- 148 **Zimdahl H, Ittrich C, Graefe-Mody U, Boehm BO, Mark M, Woerle HJ, Dugi KA.** Influence of TCF7L2 gene variants on the therapeutic response to the dipeptidylpeptidase-4 inhibitor linagliptin. *Diabetologia* 2014; **57**: 1869-1875 [PMID: 24906949 DOI: 10.1007/s00125-014-3276-y]
- 149 **Sun X, Yu W, Hu C.** Genetics of type 2 diabetes: insights into the pathogenesis and its clinical application. *Biomed Res Int* 2014; **2014**: 926713 [PMID: 24864266 DOI: 10.1155/2014/926713]

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## Basic Study

# Increased fecal viral content associated with obesity in mice

Hariom Yadav, Shalini Jain, Ravinder Nagpal, Francesco Marotta

Hariom Yadav, Department of Nutritional Biotechnology, National Agri-Food Biotechnology Institute, Mohali, Punjab 160071, India

Shalini Jain, Postgraduate Institute of Medical Education and Research, Chandigarh 160012, India

Ravinder Nagpal, Probiotics Research Laboratory, Juntendo University Graduate School of Medicine, Tokyo 1138421, Japan

Francesco Marotta, ReGenera Research group for Aging Intervention and Milano Medical, Gender Healthy Aging Unit by Genomics, 20122 Milano, Italy

**Author contributions:** Yadav H and Jain S performed majority of experiments, analyzed data and wrote manuscripts; Nagpal R and Marotta F participated significantly on study design and coordination for data analysis and interpretations, as well as writing manuscript.

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**Data sharing statement:** Data will be readily available on request.

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**Correspondence to:** Hariom Yadav, PhD, Scientist, Rama-

lingaswami Fellow, Department of Nutritional Biotechnology, National Agri-Food Biotechnology Institute, C-127, Industrial Area, Phase 8, Mohali, Punjab 160071, India. [yadavhariom@gmail.com](mailto:yadavhariom@gmail.com)  
**Telephone:** +91-301-496459  
**Fax:** +91-301-4514800

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## Abstract

**AIM:** To investigate the presence of total gut viral content in obese mice, and establish correlation with obesity associated metabolic measures and gut microbiome.

**METHODS:** Fresh fecal samples were collected from normal and obese (Leptin deficient: Lep<sup>ob/ob</sup>) mice. Total viral DNA and RNA was isolated and quantified for establishing the correlation with metabolic measures and composition of gut bacterial communities.

**RESULTS:** In this report, we found that obese mice feces have higher viral contents in terms of total viral DNA and RNA ( $P < 0.001$ ). Interestingly, these increased viral DNA and RNA content were tightly correlated with metabolic measures, *i.e.*, body weight, fat mass and fasting blood glucose. Total viral content were positively correlated with firmicutes ( $R^2 > 0.6$ ), whilst negatively correlated with bacteroidetes and bifidobacteria.

**CONCLUSION:** This study suggests the strong corre-

lation of increased viral population into the gut of obese mice and opens new avenues to explore the role of gut virome in pathophysiology of obesity.

**Key words:** Obesity; Gut; Microbiome; Virome; DNA; RNA; Virus; Fat mass

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**Core tip:** Gut microbiome is known for major constituents of bacterial population, and their association with obesity, but microbes like viruses are majorly neglected. Our investigation on the basis of hypothesis that viruses are an important part of microbial world, and found in substantial numbers into human gut, we investigated whether viral content have any correlation with obesity in mice models. Interestingly, we found that DNA and RNA viral fecal content was significantly increased in obese mice as compared to normal. This suggests that viral population may have role to regulate host metabolism and might impact obesity prevalence *via* alteration of gut microbiome composition. Our findings open a new area of research to explore the role of gut virome in obesity.

Yadav H, Jain S, Nagpal R, Marotta F. Increased fecal viral content associated with obesity in mice. *World J Diabetes* 2016; 7(15): 316-320 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v7/i15/316.htm> DOI: <http://dx.doi.org/10.4239/wjd.v7.i15.316>

## INTRODUCTION

Obesity is reaching on higher epidemic around the globe<sup>[1]</sup>. This is because of lack of successful and effective strategies to prevent and treat this health ailment. The pathophysiology of obesity is highly complex, and involves various factors, *i.e.*, genetics, environment and life style<sup>[2]</sup>. Abdominal or central obesity (abdominal fat accumulation) is strongly correlated with increased incidence of insulin resistance and metabolic syndrome<sup>[3]</sup>. Abdominal fat is in close proximity with gastrointestinal tract, and have various gut-adipose communication through various gut hormones and adipokines<sup>[4]</sup>. Recently, role of gut microbiome in obesity pathophysiology have been well established and known to play significant role in obesity progression<sup>[5]</sup>. Human and rodent studies suggest that presence of gut microbiome increases the risk of weight gain and insulin resistance<sup>[6]</sup>. Various mechanisms have been proposed to explain the role of gut-microbiome on obesity progression, *i.e.*, increased energy harvesting capacity, low grade inflammation, endotoxemia and other metabolic interferences<sup>[7]</sup>. Recently, plethora of literature has been generated to explore the role of gut microbiome (especially bacterial community)

in metabolic regulation. Therefore modulation of gut microbiome has been considered one of the important strategies to develop therapies against obesity and diabetes<sup>[7]</sup>.

Most of the studies conducted for exploring the role of gut microbiome in obesity have been focused on bacterial communities and their correlation with host metabolism<sup>[8]</sup>. Role of viruses present into gut are not investigated in relation with obesity and their impact on host metabolism and associated gut bacterial microbiome. Viruses play a critical role in maintaining bacterial population in specific environment, *i.e.*, gut, where they establish a commensal relation with their partners<sup>[9]</sup>. Specific viral particles, especially bacteriophages might play an important role in maintaining certain bacterial strains of gut microbiome, that are correlated with obesity occurrence<sup>[6]</sup>. From our best knowledge, none of the studies investigated the direct link of gut virome with obesity. We hypothesize that changes in gut viral community (gut-virome), might play an important role in maintaining and colonizing gut bacterial species that impact host metabolism. Hence establishing the role of gut-viral community on influencing gut microbiome and host metabolism will open new avenues for development of therapeutic strategies against obesity *via* targeting gut virome. In this study, we investigated the correlation between gut-virome, obesity associated metabolic measures and gut bacterial communities in mice.

## MATERIALS AND METHODS

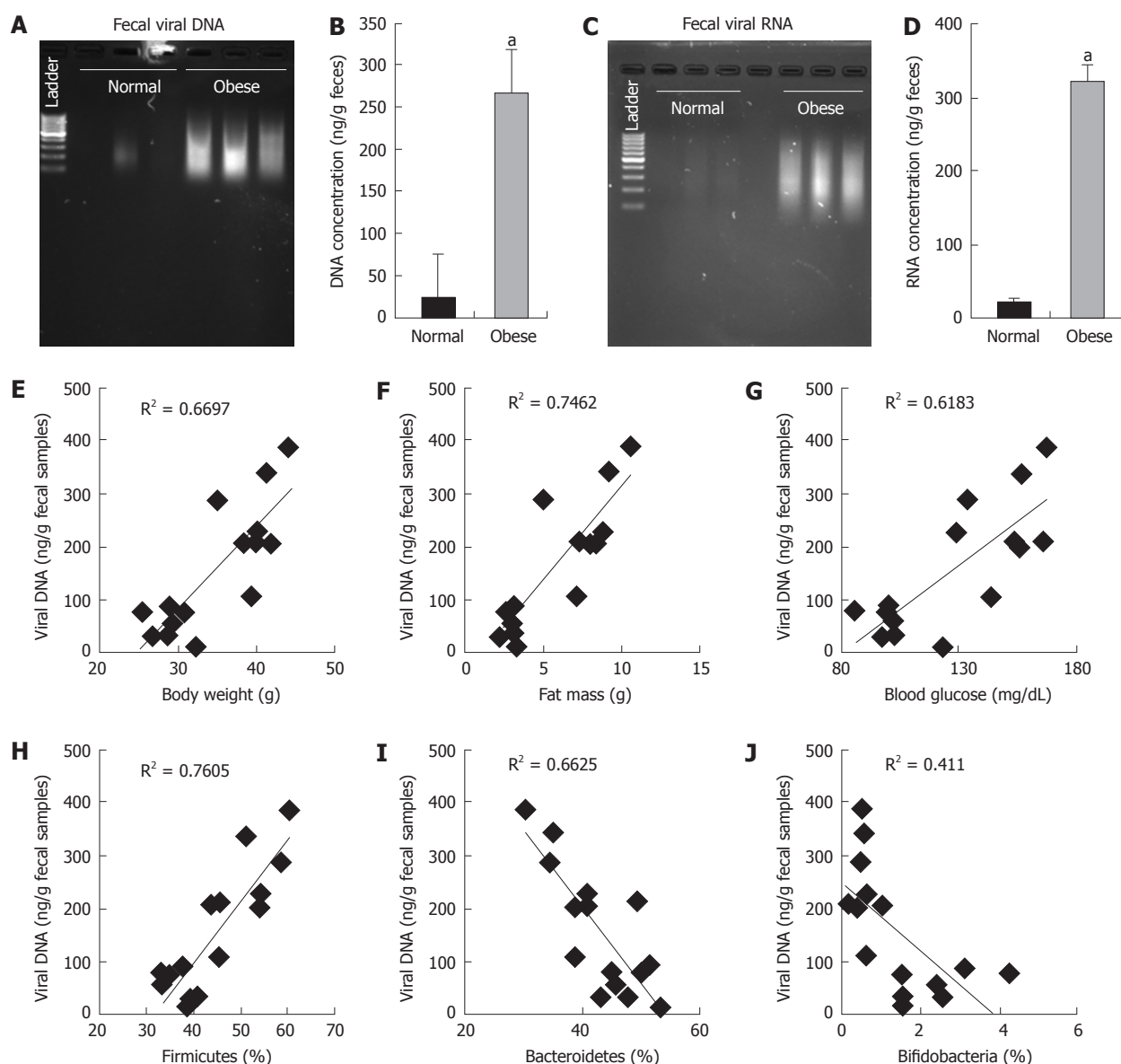
### **Animals, sample collection and biochemical analysis**

Lep<sup>ob/ob</sup> and C57J/B6 mice (male; age 6-8 wk old) were housed in a light controlled facility by maintaining 12 h light/dark cycle. Mice were maintained in identical conditions and fed with similar diet and water, *ad libitum*. Body weight was measured using a microscale balance (Cole-Parmer, IL, United States). Total fat mass was measured weighing all the major fat depots, *i.e.*, epididymal, perirenal, mesenteric, supra subscapular, anterior subcutaneous and posterior subcutaneous fat depots). Fasting (12-14 h) blood glucose was measured using Bayer Contour glucometer (Bayers Contour Diabetes Solutions, Thane, India). Fresh fecal samples were collected from each mouse by light abdominal squeezing and immediately stored in a sterile, DNase and RNase free vials at -80 °C till further use. All the animal protocols and procedures were approved by institutional animal ethics committee from University of Punjab and PGIMER, Chandigarh, India.

### **Viral DNA and RNA isolation and quantification**

Fecal viral DNA and RNA was isolated using Qiagen viral DNA and RNA isolation kits following the manufacturer's instructions. DNA and RNA quality have been checked using Agilent 2100 Bioanalyzer. Viral DNA and RNA have been quantified using NanoDrop One





**Figure 1** Fecal viral DNA and RNA content was significantly increased in obese mice and shown strong correlations with obesity associated measures and gut bacterial microbiome. A-D: Fecal viral DNA (A and B) and fecal viral RNA (C and D) content was found to be dramatically increased in obese animals; E-J: Viral DNA was positively correlated with body weight (E), fat mass (F), blood glucose (G) and firmicutes (H), whilst negatively correlated with bacteroidetes (I) and bifidobacteria (J). Values presented here are means ( $n = 7$ ) and standard error of means. Values indicated with "a" are significantly different at the level of  $P < 0.001$ .

**Table 1** Primers used for gut microbial community analysis

Gene Name	Primer sequence (5' → 3')
Universal F (Total)	TCCTACGGGAGGCAGCAGT
Universal R (Total)	GACTACCAGGTATCTAATCCTGTT
Bifidobacteria F	GCGTGCTTAACACATGCAAGTC
Bifidobacteria R	CACCCGTTTCCAGGAGCTATT
Bacteroidetes 934F	GGARCATGTGGTTTAATTTCGATGAT
Bacteroidetes 1060R	AGCTGACGACAACCATGCAG
Firmicutes 934F	GGAGYATGTGGTTTAATTTCGAAGCA
Firmicutes 1060R	AGCTGACGACAACCATGCAC

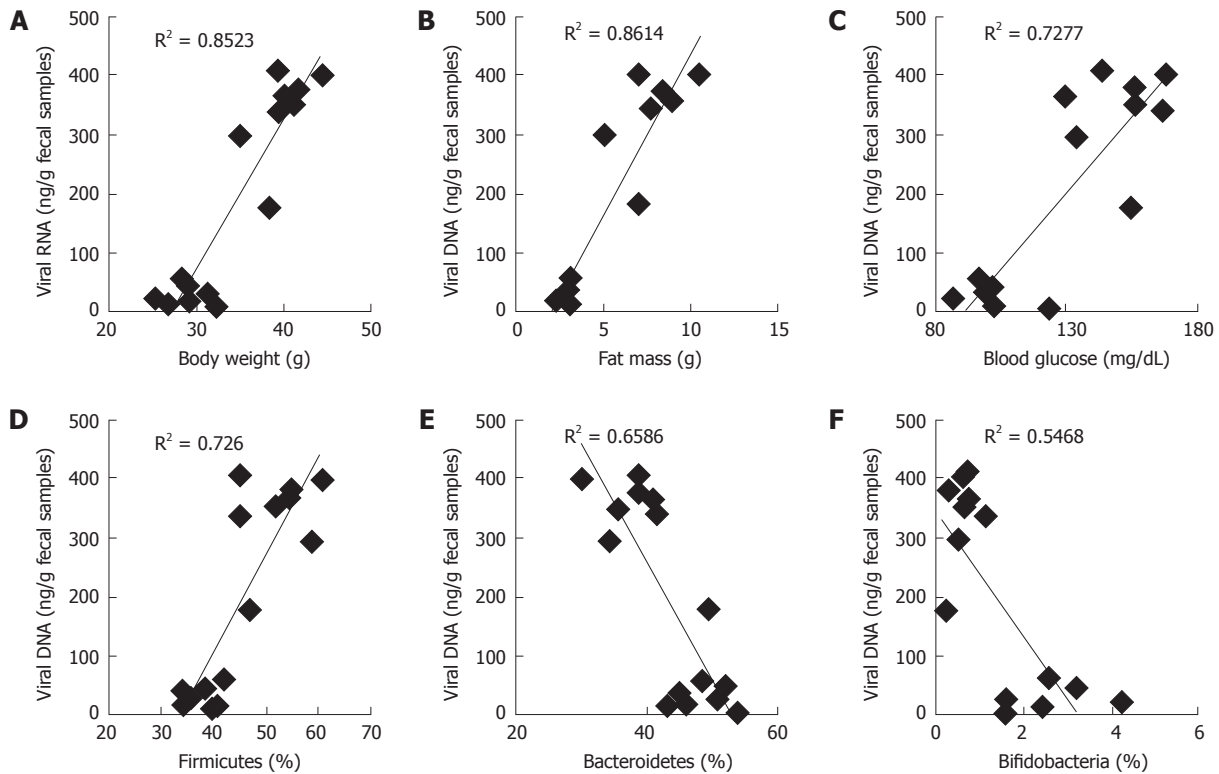
Spectrophotometer with fluorescent method (Thermo-Fisher Scientific, United States). Viral DNA and RNA quantity has been calculated nanogram per gram of fecal sample.

### Bacterial DNA quantification

Mouse total fecal DNA was isolated from separate fecal pellets than viral DNA/RNA isolation, using DNeasy kit (Qiagen). Real time PCR was performed to measure the major obesity associated bacterial community, *i.e.*, Firmicutes, bacteroidetes and Bifidobacteria using microbe specific primers (Table 1). Results are presented here as percent of bacterial DNA abundance normalized by total bacterial DNA.

### Statistical analysis

All the data expressed is mean and standard error of means. Statistical significance among the groups was calculated using two-tailed *t* test and/or one way analysis of variance, that followed by *post-hoc* tests.



**Figure 2** Fecal viral RNA content was significantly correlated with obesity associated measures and gut bacterial microbiome. A-F: Fecal viral RNA content was positively correlated with body weight (A), fat mass (B), blood glucose (C) and firmicutes (D), whilst negatively correlated with bacteroidetes (E) and bifidobacteria (F). Values presented here are means ( $n = 7$ ) and standard error of means.

Data with less than 0.05  $P$ -values considered statistically significance.

## RESULTS

Microbiome studies clearly suggest that we are surrounded by microbes, in which viruses makes a significant numbers. Around  $10^{31}$  viral particles have been estimated on earth, and human feces consist around  $10^9$  viral particles/gram<sup>[10-12]</sup>. Mammalian virome collectively called for viruses that infect eukaryotic cells (eukaryotic virome), bacterial cells (bacterial virome), archeal cells (archeal virome) and virus derived genetic elements in host chromosomes that can change host-gene expression, express proteins, or even generate infectious virus (prophages, endogenous retroviruses, endogenous viral elements)<sup>[13]</sup>. Viral infections have been associated with prevalence of obesity in animals and humans, and termed as infectobesity<sup>[14]</sup>. Considering the technological limitations for sequencing and analyzing datasets for viral communities, studies of virome has been lagged behind than bacterial microbiome. In present study, we analyzed total fecal viral content in normal vs obese (leptin deficient  $Lep^{ob/ob}$ ) mice and correlated with obesity related measures. Interestingly, we found that fecal viral DNA and RNA in obese samples was significantly higher than normal mice (Figures 1 and 2), suggesting that total DNA and RNA viral communities have been significantly increased in obese

mice. Although, our studies completely lack the types of viruses enriched in obese mice gut as compared to normal, but these very interesting observations indicate that total load of viruses have been increased in obese gut. Therefore, these results provide a strong basis to further explore the role of gut virome in obesity.

## DISCUSSION

We have observed that fecal DNA and RNA viral population positively correlated with firmicutes bacterial communities, which is known to be associated with increased obesity<sup>[5]</sup>. While viral contents were negatively correlated with bacteroidetes and bifidobacteria, that are known to be associated with lean-ness<sup>[5]</sup>. These results can be speculated in a way, that gut virome (especially bacteriophages) might have interaction with gut bacterial microbiome to modulate the bacterial species abundance in obese vs normal mice. Although, these studies gives us an intrigued and important preliminary information about the abundance of DNA and RNA viruses in obese and normal mice, but still detailed analysis to find out the types of viruses and their functionality remains completely unknown. Therefore, further studies to explore the types of viruses that are associated with increased viral DNA and RNA contents in normal vs obese mice are highly warranted.

Viruses are highly mutagenic and carries individual variations in gut viral communities have been described

earlier<sup>[9]</sup>. Viral proteins can interact with host cells and can induce biological response, *i.e.*, inflammation, receptor based cell signaling or gene expression, to modulate adipose tissue biology<sup>[15]</sup>. Hence, the results of this study also indicate that increased viral population might be contributing to release of higher amount of certain viral proteins that can interact directly with host cells to modulate metabolism and cause obesity. Therefore it will be very important to establish how these viral species and their end products (*i.e.*, proteins) are playing role in modulation of gut bacterial communities, as well as their impact on host metabolism.

## COMMENTS

### Background

Viruses have been known to infection host, bacterial and other broad array of organisms. Gut bacterial microbiome have been known to play critical role in obesity pathology, but the role of gut virome have not been explored.

### Research frontiers

The facts that viruses interacts with host cells as well as infects bacterial cells to control bacterial growth, studying virome compositions is one of the important aspects in the microbiome biology and its impact of health. Role of gut virome in obesity and gut bacterial microbiome modulation will open new frontiers of investigations.

### Innovations and breakthroughs

The authors first time have reported that total viral population have been changed in obesity mice and correlated with metabolic and gut bacterial microbiome.

### Applications

This study further open new avenues to find different types of viral populations in obese vs normal population and can develop them as a new drug targets or biomarkers.

### Terminology

Gut virome is considered as collective viral community present in fecal samples.

### Peer-review

In the current study, the authors explored the association between fecal viral content and obesity in mice. The results are significant as the fecal viral DNA and RNA content found to be elevated in obese mice model.

## REFERENCES

- 1 Farag YM, Gaballa MR. Diabetes: an overview of a rising epidemic. *Nephrol Dial Transplant* 2011; **26**: 28-35 [PMID: 21045078 DOI: 10.1093/ndt/gfq576]
- 2 Wild SH, Byrne CD. ABC of obesity. Risk factors for diabetes and coronary heart disease. *BMJ* 2006; **333**: 1009-1011 [PMID: 17095784 DOI: 10.1136/bmj.39024.568738.43]
- 3 Brunzell JD, Hokanson JE. Dyslipidemia of central obesity and insulin resistance. *Diabetes Care* 1999; **22** Suppl 3: C10-C13 [PMID: 10189557]
- 4 Yi CX, Tschöp MH. Brain-gut-adipose-tissue communication pathways at a glance. *Dis Model Mech* 2012; **5**: 583-587 [PMID: 22915019 DOI: 10.1242/dmm.009902]
- 5 Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. *Nature* 2006; **444**: 1022-1023 [PMID: 17183309]
- 6 Turnbaugh PJ, Backhed F, Fulton L, Gordon JI. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host Microbe* 2008; **3**: 213-223 [PMID: 18407065 DOI: 10.1016/j.chom.2008.02.015]
- 7 Delzenne NM, Neyrinck AM, Backhed F, Cani PD. Targeting gut microbiota in obesity: effects of prebiotics and probiotics. *Nat Rev Endocrinol* 2011; **7**: 639-646 [PMID: 21826100 DOI: 10.1038/nrendo.2011.126]
- 8 Cani PD. Gut microbiota and obesity: lessons from the microbiome. *Brief Funct Genomics* 2013; **12**: 381-387 [PMID: 23616309 DOI: 10.1093/bfpg/elt014]
- 9 Sime-Ngando T, Lucas S, Robin A, Tucker KP, Colombet J, Bettarel Y, Desmond E, Gribaldo S, Forterre P, Breitbart M, Prangishvili D. Diversity of virus-host systems in hypersaline Lake Retba, Senegal. *Environ Microbiol* 2011; **13**: 1956-1972 [PMID: 20738373 DOI: 10.1111/j.1462-2920.2010.02323.x]
- 10 Rohwer F. Global phage diversity. *Cell* 2003; **113**: 141 [PMID: 12705861]
- 11 Schoenfeld T, Liles M, Wommack KE, Polson SW, Godiska R, Mead D. Functional viral metagenomics and the next generation of molecular tools. *Trends Microbiol* 2010; **18**: 20-29 [PMID: 19896852 DOI: 10.1016/j.tim.2009.10.001]
- 12 Suttle CA. Viruses in the sea. *Nature* 2005; **437**: 356-361 [PMID: 16163346]
- 13 Virgin HW, Wherry EJ, Ahmed R. Redefining chronic viral infection. *Cell* 2009; **138**: 30-50 [PMID: 19596234 DOI: 10.1016/j.cell.2009.06.036]
- 14 Dhurandhar NV. Infectobesity: obesity of infectious origin. *J Nutr* 2001; **131**: 2794S-2797S [PMID: 11584109]
- 15 Ponterio E, Gnessi L. Adenovirus 36 and Obesity: An Overview. *Viruses* 2015; **7**: 3719-3740 [PMID: 26184280 DOI: 10.3390/v7072787]

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*World Journal of Diabetes*  
Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China  
Telephone: +86-10-85381891  
Fax: +86-10-85381893  
E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
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## Assessment of the cardiovascular and gastrointestinal autonomic complications of diabetes

Christina Brock, Birgitte Brock, Anne Grave Pedersen, Asbjørn Mohr Drewes, Niels Jessen, Adam D Farmer

Christina Brock, Anne Grave Pedersen, Asbjørn Mohr Drewes, Adam D Farmer, Mech-Sense, Department of Gastroenterology and Hepatology, Aalborg University Hospital, DK-9000 Aalborg, Denmark

Christina Brock, Department of Pharmacotherapy and Development, University of Copenhagen, DK-2450 Copenhagen, Denmark

Christina Brock, Anne Grave Pedersen, Clinical Biochemistry, Aarhus University, DK-3780 Aarhus, Denmark

Niels Jessen, Department of Clinical Medicine, Aarhus University, DK-3780 Aarhus, Denmark

Adam D Farmer, Department of Gastroenterology, University Hospitals of North Midlands, Staffordshire WS73 JQ, United Kingdom

Adam D Farmer, Centre for Digestive Diseases, Wingate Institute of Neurogastroenterology, Blizard Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London EN1 1NX, United Kingdom

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**Correspondence to:** Christina Brock, DVM, PhD, Associate

Professor, Mech-Sense, Department of Gastroenterology and Hepatology, Aalborg University Hospital, Mølleparkvej 4, DK-9000 Aalborg, Denmark. [christina.brock@rn.dk](mailto:christina.brock@rn.dk)  
Telephone: +45-9766-0510  
Fax: +45-9766-3577

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### Abstract

The global prevalence of diabetes mellitus is increasing; arguably as a consequence of changes in diet, lifestyle and the trend towards urbanization. Unsurprisingly, the incidence of both micro and macrovascular complications of diabetes mirrors this increasing prevalence. Amongst the complications with the highest symptom burden, yet frequently under-diagnosed and sub-optimally treated, is diabetic autonomic neuropathy, itself potentially resulting in cardiovascular autonomic neuropathy and gastrointestinal (GI) tract dysmotility. The aims of this review are fourfold. Firstly to provide an overview of the pathophysiological processes that cause diabetic autonomic neuropathy. Secondly, to discuss both the established and emerging cardiometric methods for evaluating autonomic nervous system function *in vivo*. Thirdly, to examine the tools for assessing pan-GI and segmental motility and finally, we will provide the reader with a summary of putative non-invasive biomarkers that provide a pathophysiological link between low-grade neuro inflammation and diabetes, which may allow earlier diagnosis and intervention, which in future may improve patient outcomes.

**Key words:** Autonomic nervous system; Gastrointestinal dysmotility; Neuroinflammation; Biomarkers; Diabetic

neuropathy

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**Core tip:** Autonomic complications are common and bothersome long-term sequelae of diabetes. However, they are frequently under-diagnosed and sub-optimally treated. Arguably this is as a consequence of a lack of appreciation of the various testing options that are available, particularly for end organ dysfunction such as within the cardiovascular and gastrointestinal systems. Our review aims to provide a succinct review of the current investigational armamentarium that are available and also provide the reader with a summary of the cutting edge techniques that have the potential to influence clinical practice in the future.

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## INTRODUCTION

The world prevalence of diabetes among adults will be 7.7%, affecting 439 million adults by 2030. Between 2010 and 2030, there will be a 69% increase in numbers of adults with diabetes in developing countries and a 20% increase in developed countries<sup>[1]</sup>. This epidemic is potentially a consequence of changes in diet, lifestyle and the trend towards urbanization. Diabetes is associated with significant economic burden with healthcare costs estimated to be in the order of \$132 billion annually in the United States and £10 billion in the United Kingdom<sup>[2,3]</sup>. Unsurprisingly, the prevalence of complications of diabetes reflects the increases in prevalence. Arguably amongst the most burdensome from a symptomatic point of view, yet frequently under-diagnosed, is the neuropathy that causes dysfunction of the autonomic nervous system (ANS), referred to as diabetic autonomic neuropathy (DAN), itself potentially leading to myriad of complications frequently manifest in the cardiovascular system and gastrointestinal (GI) tract. In addition to the bothersome nature of symptoms, Ewing *et al*<sup>[4]</sup> reported that in those with DAN, the survival rate at 5 years following diagnosis is as low as 47%.

The ANS is a bi-directional hierarchically controlled brain body interface that serves to integrate and modulate the internal milieu in response to the external environment thereby serving to maintain homeostasis. The ANS consists of the enteric nervous system and two broadly opposing branches referred to as the sympathetic (SNS) and parasympathetic nervous systems (PNS), having ubiquitous innervation throughout the body. The overall aim of this paper is to provide the reader with a

contemporaneous and succinct review of the assessment of the autonomic complications of diabetes and discuss potential future biomarkers.

## DIABETIC AUTONOMIC NEUROPATHY

One of the major microvascular complications of diabetes is development of neuropathy, defined as "the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes", which may deleteriously affect sensory, motor and autonomic nerve fibers<sup>[5]</sup>.

Diabetes induced sensory and motor neuropathies affects C-fibers first and then progressively symmetrical affection of thick (A $\beta$ ) and thin (A $\delta$ ) - fiber neuropathy influencing axons of the distal lower extremities in a "glove and stocking" distribution. Interestingly, that despite comparable traditional risk factors, Asians with diabetes have substantially less large and small fiber neuropathy in comparison to matched Europeans<sup>[6]</sup>. Whilst clinicians would readily recognize that such symptoms represent a sensory neuropathy, symptoms related to DAN are often under appreciated, recognized and investigated. Diabetic sensorimotor polyneuropathies can be categorized according to the Toronto classification (Table 1)<sup>[5]</sup>. The traditional view of the pathogenesis of DAN is considered to be sequelae of vascular compromise, it has been more recently proposed that such complications represent the progression of systemic capillary dysfunction, which frequently are already present at diagnosis in those with type 2 diabetes<sup>[7]</sup>. Moreover, distinct differences in haemodynamic properties within the epineurium of *e.g.*, the sural nerve, have also been proposed to play an important role in the pathogenesis of painful diabetic neuropathy<sup>[8]</sup>. However, the order of these factors in the aetiopathogenesis of diabetic neuropathy has been challenged by Danish researchers, as changes in endoneurial capillary morphology and vascular reactivity apparently may predate the development of diabetic neuropathy in humans<sup>[7]</sup>.

Considering that the pathophysiology is largely similar, DAN can be regarded as an entity not dissimilar to the aforementioned peripheral neuropathy<sup>[5]</sup>. DAN can be usefully regarded as both a structural and/or a metabolic disorder, and the clinical manifestation of which can be present with or without the presence of large fiber neuropathy. DAN may affect cardiovascular, GI sensorimotor, urogenital systems, and sudomotor function. The presence of DAN confers a heightened risk of mortality in diabetes and frequently co-exists with other peripheral polyneuropathies<sup>[9]</sup>. Evidence suggests that subclinical DAN can occur within the first year of onset of type 2 diabetes (T2DM), and within two years in type 1 diabetes (T1DM), although often unrecognized for a number of years after their onset<sup>[10]</sup>.

Nevertheless, the formal diagnosis of DAN is frequently delayed, the causes of which are most certainly multifactorial but arguably includes the non-specificity of presenting symptoms, the lack of clinician appreciation

**Table 1** Toronto classification of diabetic sensory neuropathy<sup>[5]</sup>

Definition of minimal criteria for diabetic sensorimotor polyneuropathy	Clinical features
Possible	Reduced sensation, positive neuropathic sensory symptoms (burning pain in the distal lower extremities), symmetrical reduction in distal sensation and/unequivocally decreased or absent ankle reflexes
Probable	A combination of two or more of the following: Neuropathic symptoms, decreased distal sensation, or unequivocally decreased or absent ankle reflexes
Confirmed	Decreased nerve conduction on objective testing with signs and symptoms as above
Subclinical	Decreased nerve conduction on objective testing in the absence of signs or symptoms

and the limited availability of specialized diagnostic services. Cardiovascular autonomic neuropathy is frequent, which can result in life threatening complications such as arrhythmias, silent myocardial ischemia and sudden death. However, DAN can potentially affect any portion of the ANS, and should therefore be considered a systemic disorder<sup>[11]</sup>. Evidence suggests that up to 10% of those with diabetes are at risk of developing DAN which may manifest as a variety of troublesome symptoms including orthostatic hypotension, aberrant GI motility and erectile dysfunction all of which can lead to a diminution in quality of life<sup>[5]</sup>.

Hitherto, the focus of assessment for DAN has been derived from measures such as heart rate variability (HRV) and sudomotor function. However, over the recent past there have been considerable advances in measuring the “downstream” effects of DAN on both the cardiovascular system and the GI tract.

## **PATHOPHYSIOLOGY OF DIABETIC AUTONOMIC NEUROPATHY**

### ***Hyperglycemia induced macro- and microvascular complications***

After 20 years of diabetes, neuropathy can be objectively demonstrated in up to 40%-50%<sup>[12]</sup>. The pathophysiology of neuropathy is multifactorial with structural and metabolic alterations having been described within axons, Schwann cells, and microvascular elements within the endoneurium and extracellular matrices<sup>[13]</sup>. Newer findings suggest that changes in the endoneurial capillary morphology and vascular reactivity are present before development of diabetic neuropathy in humans<sup>[7]</sup>. In addition, the authors found an association between the level of endoneurial hypoxia and reductions in nerve conduction velocity, in diabetes patients with manifest neuropathy.

Using experimental models of diabetes, reduced levels of neurotrophic support, including nerve growth factor and insulin like growth factor, have been implicated in reducing endoneurial blood flow thereby leading to neuronal damage<sup>[14]</sup>. In addition, such impairments in blood flow also result in alterations in Na<sup>+</sup>/K<sup>+</sup> ATP-ase activity and nitric oxide metabolism. Animal studies suggest that altered Na<sup>+</sup>/K<sup>+</sup> pump function may occur due to C-peptide deficiency, resulting in the shunting of glucose through the polyol pathway, thereby leading to increased

levels of sorbitol and alterations of the nerve excitability recovery cycle which further contribute to neuronal damage<sup>[15,16]</sup>.

Peripheral and autonomic neurons, as well as their interconnections, are particularly vulnerable to hyperglycemia<sup>[17]</sup>. The mechanisms that underlie this vulnerability can be considered to both direct, as a consequence of heightened influx of extracellular glucose and indirectly through a plethora of other biochemical pathways. Examples of such indirect metabolic pathway are summarized in Figure 1 and include, but are not limited to, the following.

**Polyol pathway:** In the polyol pathway intracellular glucose is converted to sorbitol by the rate limiting enzyme aldose reductase, in an energy dependent manner *via* nicotinamide adenine dinucleotide phosphate<sup>[18]</sup>. The activation of this pathway may result in osmotic damage and diminution of Na<sup>+</sup>/K<sup>+</sup>-ATPase activity<sup>[19]</sup>. These processes lead to increased intracellular oxidative stress<sup>[20]</sup>.

**Hexosamine pathway:** The hexosamine biosynthesis pathway is a minor branch of glycolysis, where fructose-6-phosphate is converted to glucosamine-6-phosphate, catalyzed by the rate-limiting enzyme: Glutamine: Fructose-6-P-amidotransferase.

**Formation of reactive oxygen species:** In diabetes, reactive oxygen species (ROS) play an important role in the development of cardiovascular diseases, through excessive formation of oxidants, decreased bioavailability of nitric oxide, and decreased antioxidant capacity in the vasculature and kidneys<sup>[21]</sup>. These processes are initiated and amplified during chronic hyperglycemic conditions<sup>[22]</sup>.

**Increased diacylglycerol and protein kinase C pathways:** Increased activation of the polyol pathway may cause a decrease in the activity of (Na<sup>+</sup>/K<sup>+</sup>) ATPase, and studies have suggested that this drop may activate diacylglycerol and protein kinase C (PKC) pathways<sup>[23]</sup>. Activation of PKC pathways increase cytosolic phospholipase A2 activity and produces a pro-inflammatory mediators such as prostaglandin E<sub>2</sub>, which inhibits cellular (Na<sup>+</sup>/K<sup>+</sup>) ATPase<sup>[24]</sup>.

**Formation of advanced glycation end products:** Hyperglycaemia results in the formation of advanced glycation end products, comprising of proteins or lipids



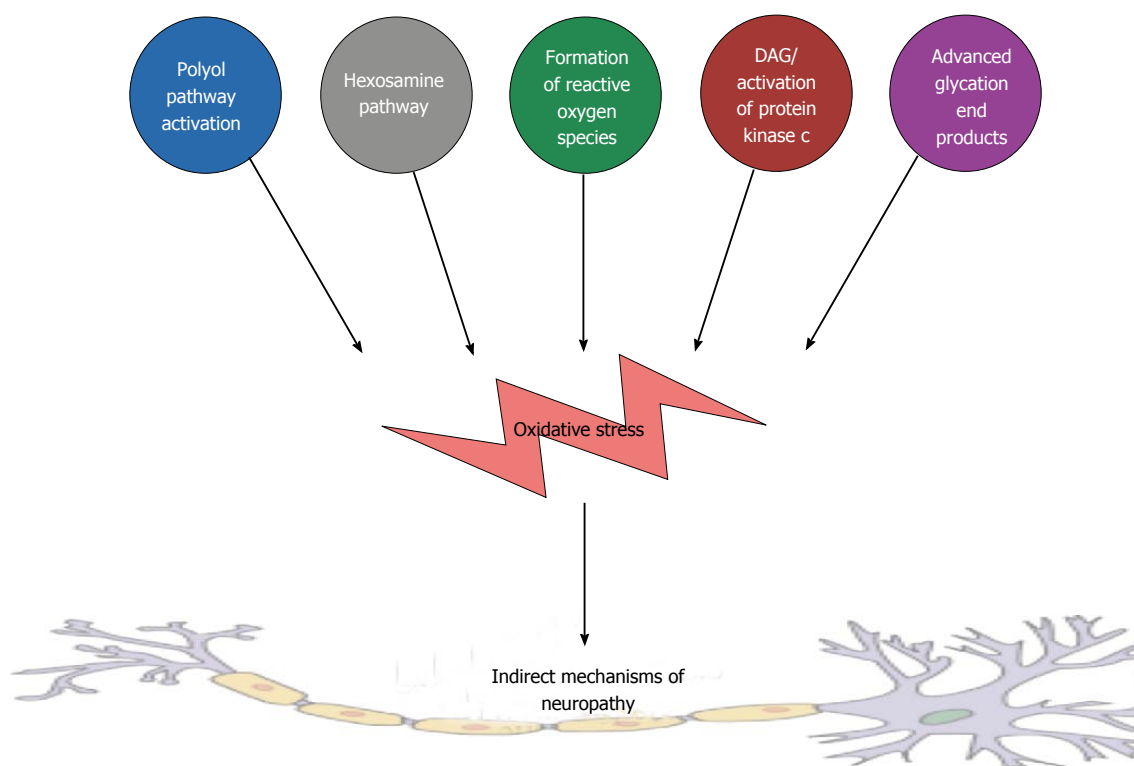


Figure 1 A highly schematic summary of the proposed indirect mechanisms of neuropathy. DAG: Diacylglycerol.

that become glycosylated after prolonged exposure to sugars<sup>[25]</sup>. This results in a diminished redox capacity of the neuron leading to enhanced vulnerability to ROS.

Cumulatively, these biochemical pathways, in conjunction with activation of the complement system<sup>[26]</sup>, coalesce to form a cumulative indirect cascade that can initiate and summate neuro-inflammation, as is observed in DAN.

## ASSESSMENT OF AUTONOMIC DYSFUNCTION

The last three decades have witnessed the increasing recognition of the pivotal role of the ANS in the pathophysiology of a number of disorders including diabetes. Although ANS function can be measured directly, using a needle recording of the peroneal nerve for instance, such methods are invasive and time consuming. Therefore, indirect, or proxy measures of ANS function have been developed, the most popular and widely utilised being HRV and are summarised in Figure 2.

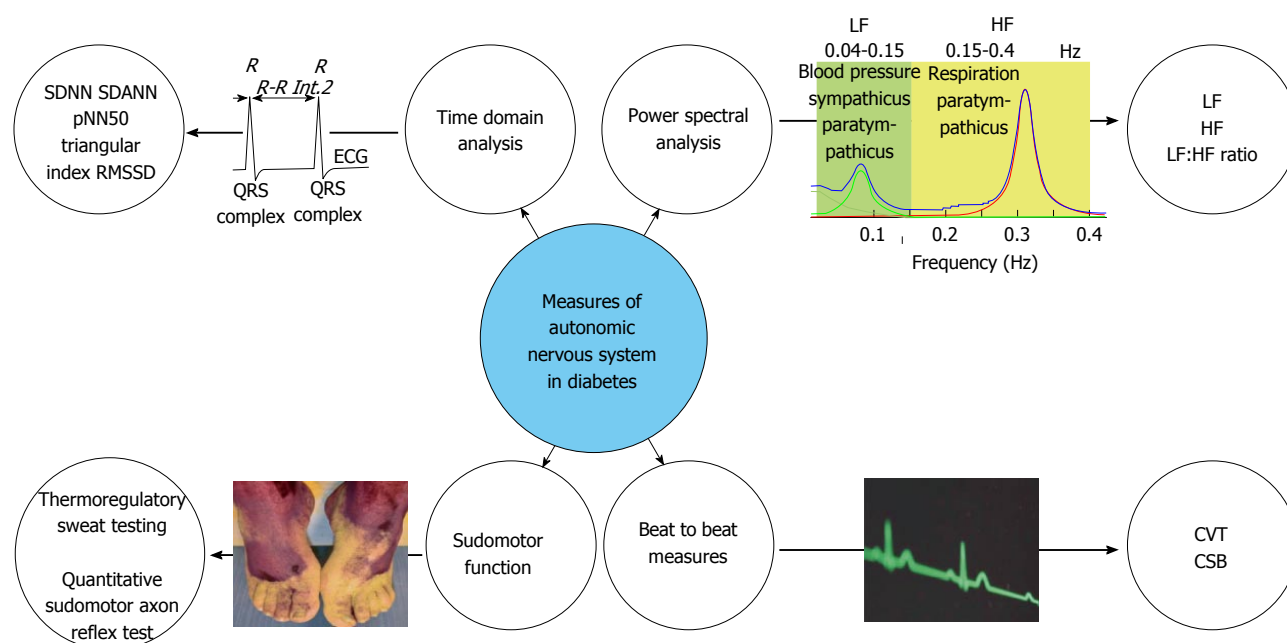
## HEART RATE VARIABILITY

The clinical relevance of HRV was first appreciated in 1965 when Lee *et al*<sup>[27]</sup> demonstrated that foetal distress was preceded by alterations in the inter-beat intervals between successive R waves in the electrocardiogram (ECG), before any appreciable changes occurred in heart rate (HR) *per se*. This epiphenomenon in the oscillations in the interval between successive heartbeats is known

as "heart rate variability". In deriving physiologically salient measures from HRV, there are three broad methods, time domain, power spectral analysis and beat-to-beat measures.

## TIME DOMAIN ANALYSIS OF HEART RATE VARIABILITY

Since HR is controlled within a negative feedback loop influenced by both the SNS and PNS, the examination of beat-to-beat periodicities can provide an insight into their relative influences. Such variations in HR may be examined using time domain analysis. In a continuous ECG recording, the interval between consecutive normal QRS complexes on the ECG is known as the normal-to-normal (NN) interval. From the NN interval, statistical time domain measures can be derived and are divided into two classes, firstly those derived from the direct measurement of NN intervals and secondly those derived from the difference between NN intervals. The simplest variable is the standard deviation of normal-to-normal (SDNN RR intervals), which reflects the cyclic components of variability within the recording. Other commonly used measures are detailed in Table 2. The major disadvantage of these methods is the limited statistical power for the evaluation of short-term recordings of less than five minutes. Time domain analysis has been widely used to characterize autonomic neuropathy in diabetes, and has shown to be associated with the degree of sensorimotor neuropathy and also influences symptom generation peripherally within the GI tract<sup>[28]</sup>. Finally, reduced HRV



**Figure 2** A summary of the autonomic testing options. SDNN: Standard deviation of normal-to-normal; ECG: Electrocardiogram; HF: High frequency; LF: Low frequency.

**Table 2** Time domain analysis variables and their physiological relevance

Variable (units)	Description	Physiological relevance
SDNN (ms)	Standard deviation of the normal RR (NN) interval reflecting all of the cyclic components responsible for variability in the period of recording	An overall estimate of HRV, but does not indicate the contribution of any particular influence
SDANN (ms)	Standard deviation of the averages of NN intervals calculated over a short period of time, usually less than five minutes	Reflects the influence of circadian rhythms on autonomic function
pNN50 (%)	The proportion of NN intervals having a difference of > 50 ms	Reflects predominant vagal influence on variability
Triangular index (ms)	The integration of the density distribution of all the NN intervals as a function of the maximum density	Overall estimate of HRV similar to SDNN
RMSSD (m/s)	The square root of the means squared differences in successive NN intervals	Estimate of the short-term components of HRV

SDNN: Standard deviation of normal-to-normal; NN: Normal-to-normal; HRV: Heart rate variability.

also were associated with altered central processing within the operculum-insular network, underlining the systemic influence of diabetic neuropathy<sup>[29]</sup>.

## POWER SPECTRAL ANALYSIS OF HEART RATE VARIABILITY

The ANS activity that influences HRV is periodic in its nature, with sympathetic and parasympathetic components oscillating at distinct frequencies. The purpose of the frequency domain analysis of HRV (spectral analysis) is to dissect HRV into its specific frequency components, which defines the energy per unit time, which is often referred to as "power", contained in each frequency component. Power spectral analysis has become the prevailing model for exploring HRV, and therefore autonomic function, within the literature. When considering short-term recordings obtained in resting conditions, the HRV spectrum is characterised by three major components at high (HF), low (LF) and

very low frequency. The HF band represents respiratory sinus arrhythmia, as this is generally considered to represent vagal output to the heart, and this is termed cardiac vagal control, whereas the LF band is considered to represent sympathetic activity. Thus by examining the ratio between LF and HF power, sympathovagal balance can be derived. However, there are a number of methodological challenges of using HRV, notwithstanding assumptions concerning a relative constant respiratory rate and depth, referred to as respiratory stationarity, and limited temporal resolution such that these measures are not validated for time epochs of less than five minutes<sup>[30,31]</sup>. Such a shift in the sympathovagal balance has been proposed to be the underlying mechanism of symptom improvement in patients suffering from gastroparesis, who were treated with gastric electrical stimulation.

## BEAT-TO-BEAT MEASURES

In attempting to overcome these methodological chall-

enges, beat-to-beat measures of ANS “tone” have been recently developed and validated such as cardiac vagal tone and cardiac sensitivity to the baroreflex which measure efferent and afferent vagal tone respectively. In a preliminary study, we have demonstrated in 14 T1DM patients that lower cardiac vagal tone and cardiac sensitivity to the baroreflex were associated with disease duration, which was independent of glycaemic control and age<sup>[32]</sup>. Therefore, such novel autonomic indices may offer a longitudinal biomarker, which may aid in the prediction of autonomic neuropathy. Given their relative ease of use, and the lack of need for expert interpretation, these parameters could be useful as near patient screening tools in the future.

## SUDOMOTOR FUNCTION

Patients with diabetic neuropathy typically have decreased sweating in the feet, which is associated with dry skin, itching and foot ulceration. Sweat glands are innervated by the sudomotor, postganglionic, unmyelinated cholinergic sympathetic C-fibers. Several methods have been developed to assess sudomotor function and contribute to the detection of autonomic dysfunction in diabetic peripheral neuropathy. The thermoregulatory Sweat testing (TST) evaluates the integrity of central and peripheral sympathetic sudomotor pathways<sup>[33]</sup>. The core body temperature is artificially raised to 38 °C, by increasing the ambient room temperature within a chamber, and a maximal sweat response is detected by a change in an indicator dye colour. Abnormal sweating patterns can therefore be recorded and provides a general index of severity of the autonomic failure. Nevertheless TST is limited by the fact that it cannot differentiate pre- from post-ganglionic lesions, is time consuming, requires special equipment, research facilities and patient preparation<sup>[33]</sup>. The quantitative sudomotor axon reflex test (QSART) evaluates postganglionic sympathetic cholinergic sudomotor function. Sweat glands are stimulated with a cholinergic agent and the sweat production is measured as an increase of humidity through a hygrometer. QSART is capable of detecting neuropathy with a sensitivity of > 75%<sup>[5]</sup>. However, QSART is unable to detect pre-ganglionic lesions, requires special equipment and is not widely available<sup>[5]</sup>. By combining QSART with TST sensitivity is improved to 98% and it furthermore provides the clinician with the possibility to localize the lesion<sup>[34]</sup>. A relatively novel non-invasive rapid screening test, the Sudoscan, has been introduced, which provides sensitivity of 65% and specificity of 80% in correct classification of DAN. Furthermore, the test showed strong association between foot and hand electric skin conductance and nerve conduction tests<sup>[35]</sup>. Another user-friendly technique is the visual indicator test, referred to as the Neuropad. The Neuropad has high sensitivity but moderate specificity against large fibre neuropathy assessments. However the receiver operator characteristics of Neuropad is significantly improved,

when used in combination with corneal nerve fibre length (< 14 mm/mm<sup>2</sup>) with a sensitivity and specificity of 83% and 80%, respectively<sup>[36]</sup>.

## ASSESSMENT OF GASTROINTESTINAL TRACT DYSFUNCTION

GI symptoms, maybe divided into those arising in the foregut, including the oesophagus and stomach, and those limited to the mid and hindgut. Although intuitively, considering that diabetes is a systemic disorder, a considerable degree of overlap between these three distinct anatomical areas would be expected. Up to 50% of patients with diabetes have experienced disabling GI symptoms, including nausea, vomiting, bloating, early satiety, and abdominal pain and are thought to be sequelae of GI dysmotility<sup>[37]</sup>. GI dysmotility includes delayed gastric emptying, gastroparesis, rapid gastric emptying and other motor dysfunctions, such as impaired distention within the gastric fundus.

Gastroparesis, *i.e.*, the pathological delay in the emptying of contents from the stomach into the small bowel, is one of the most frequently encountered GI complications. However, the degree of gastric emptying and symptom burden is often poorly correlated<sup>[38]</sup>. Up to 12% of patients with diabetes report symptoms consistent with GI dysmotility and such symptoms may result in nutritional compromise, diminished quality of life and poor glycaemic control as a result of impaired nutritional delivery into the small bowel<sup>[39]</sup>.

GI motility is regulated and coordinated in a complex bidirectional interaction between the central nervous system, the ANS, the enteric nervous system and various endocrine and hormonal pathways. As its name suggests, the vagus nerve, which innervates the entire GI tract, apart from the distal third of the colon, has a stimulatory effect on the enteric nervous system and thus enhances GI motility, an effect that is broadly antagonized by sympathetic fibres. The interplay between these and changes in cellular level is largely unknown, but the pathophysiological mechanisms leading to gastroparesis are multifactorial in nature. However, the ANS is likely to be of critical importance. For instance, similar GI symptoms to those reported by patients with diabetes are seen in non-diabetics following truncal vagotomy, a previously frequently used surgical intervention for peptic ulcer disease in the pre-proton pump inhibitor era. These observations gave rise to the initial assumption that gastric dysmotility reflects irreversible damage to the vagal nerve. Currently, as there is a paucity of investigations to directly assess GI autonomic function directly, *vide infra*, the evaluation of cardiometrically derived autonomic function is often used as surrogate marker of the function of the abdominal vagus. However, the reported correlations to date have been relative weak, and in other studies, no relationship between gastric emptying and autonomic function has been

Table 3 A comparison of the various contemporaneous techniques for the measurement of gastrointestinal motility <sup>[43]</sup>									
Technique	Area of the GI tract evaluated	Length of stay required in clinic/office	Acceptability to the patient	Radiation exposure	Physiological conditions of measurement	Standardization of test	Measurement of propagating contractions	Availability/expense of test	Ease of interpretation of the result
Gastric emptying scintigraphy	Stomach	c.5 h	High	Yes	Yes	No	No	Widely/moderately expensive	Moderate
Whole gut scintigraphy	Pan-GI	c.8 h	High	Yes	Yes	Yes	No	Very limited/very expensive	Difficult
Radio - opaque marker study	Stomach colon	30 min to c.4 h	High	Yes	Yes	No	No	Widely/inexpensive	Easy
<sup>13</sup> C octanoic acid breath test	Stomach		High	No	Yes	Yes	No	Very limited/inexpensive	Relatively easy
Wireless motility capsule	Pan-GI	c.30 min	High	None	Yes	Yes	No	Limited/currently moderately expensive	Relatively easy

GI: Gastrointestinal.

shown<sup>[40]</sup>. The prevalence GI dysmotility is likely to be associated with the duration of diabetes, and thus attributable to an increased prevalence of autonomic neuropathy. The prevalence of GI dysmotility, and specifically gastroparesis, also appears to be higher in females than in males for uncertain reasons, but potentially suggesting a hormonal effect on the disease process.

As mentioned, GI symptoms *per se* whilst occurring frequently in diabetes are not strongly predictive of physiological abnormalities on objective testing. Therefore, the use of patient reported tools is insufficient to establish a formal diagnosis but are useful in establishing symptom severity. There are a number of methods for objectively evaluating GI motility and these are summarised in Table 3.

### SCINTIGRAPHIC ASSESSMENT OF GASTROINTESTINAL MOTILITY

#### Scintigraphic gastric emptying

The current gold standard for the diagnosis of diabetic gastroparesis is the scintigraphic evaluation of gastric emptying<sup>[38]</sup>. It is generally recommended that prior to testing patient should (1) have serum glucose levels that are stable; (2) avoid medications that influences gastric emptying for 48-72 h prior to testing; and (3) avoid nicotine exposure during the test period as these factors can confound interpretation of the test results. Patients are fed a standardized meal of <sup>99m</sup>Tc-sulfur colloid-containing eggs, following which serial imaging over 4 h is undertaken using a gamma camera. Gastroparesis is considered to be present if > 60% of the isotope activity remains in the stomach 2 h after the test ingestion - or if at least 10% of the initial activity is still detected after 4 h<sup>[37]</sup>.

#### Scintigraphic small bowel and colonic motility

Small bowel and colonic transit can be measured in a similar manner to gastric emptying, although serial imaging is prolonged. Delayed small bowel transit is diagnosed if < 40% of total small bowel of the isotope activity has accumulated in the terminal ileum-cecum at 6 h. To assess colonic transit, images of the colon are acquired at 24, 48, and 72 h after ingestion of the radiolabelled meal, with subsequent calculation of a metric referred to as the geometric centre. The geometric centre is an average of the intra colonic, weighted by segment colonic region, and intra-faecal distribution of the isotope.

However, scintigraphy is relatively expensive, associated with radiation exposure and still not standardized across centres. Moreover, due to significant radiation burden, scintigraphy limits its application in children, women of child bearing potential, and subjects undergoing repetitive measurements of gastric emptying in a short period of time.



## RADIO-OPAQUE MARKERS

An alternative method for measuring GI motility is to use indigestible radio-opaque markers (ROMs) coupled with standard radiography/fluoroscopy.

### *ROM gastric emptying*

ROM is given together with a standard meal. Emptying of ROM is followed with fluoroscopy every hour until all ROMs are emptied or for a maximum of 6 h. When compared to scintigraphic method, the ROM method in diabetic patients has comparable specificity albeit with less sensitivity<sup>[41]</sup>. In other words, this means a normal ROM test does not exclude delayed gastric emptying, and if the clinical suspicion of gastroparesis remains, scintigraphy should be performed. However, the ROM method may represent a reasonable "screening test" for delayed gastric emptying as it is inexpensive and a widely available.

### *Radio-opaque marker colonic transit*

Colonic transit can be measured using a ROM technique, although there is a current lack of standardization, for instance more than 10 different testing protocols have been published. In broad terms, a patient ingests a known quantity of ROM and then subsequent has a plain abdominal radiograph undertaken at a defined time point, usually a number of days post ingestion, to define whether the transit is normal or delayed.

## GASTRIC EMPTYING BREATH TESTING

Gastric emptying tests use non-radioactive forms of carbon incorporated in safely ingestible food or liquid products. The substrate is <sup>13</sup>C-octanoic acid, which is labelled to a standardized meal, which is absorbed in the small intestine and metabolized to <sup>13</sup>CO<sub>2</sub>, which is then expelled from the lungs during respiration. The rate-limiting step in this conversion is gastric emptying. The breath test correlates well with scintigraphic findings, it has been proposed as a non-invasive, reliable test for measuring gastric emptying, without recourse to the use of ionizing radiation<sup>[42]</sup>. Additionally, the breath test is less expensive and easier to perform than scintigraphy and offers the added advantage of being able to be undertaken in the office environment and shipped to a laboratory for analysis<sup>[42]</sup>.

## WIRELESS MOTILITY CAPSULE

The wireless motility capsule (WMC) is an indigestible single-use capsule which provides a further option in which gastric emptying, small bowel transit and colonic transit times can be concurrently measured<sup>[43]</sup>. The WMC consists of a wireless transmitting capsule, a portable receiver worn by the patient for the duration of the test as well as analysis software. Following an overnight fast, the patient consumes a standardized meal of known fat and calorific content, which initiates postprandial motility

patterns. Immediately after the meal, the patient ingests the WMC after which they are free to leave the clinical setting.

The WMC records pH, pressure and temperature as it transverse the GI tract. Gastric emptying time is reflected by an abrupt change in pH as the capsule moves from the acidic environment of the stomach to the alkaline environment of the duodenum. Small bowel transit time is the time from exit of the stomach to an abrupt pH drop of at least 1 pH unit around the ileocaecal junction. Colonic transit time is defined as time between caecal entry of capsule and its exit from the body. The whole gut transit time is the combined transit time of gastric emptying time, small bowel transit time and colonic transit time and is defined as delayed when greater than 73 h (Figure 3)<sup>[44]</sup>. Thus the WMC offers a minimally invasive alternative to the measurement of regional and whole gut transit. The capsule does not require any radiation, is standardized and can be carried out in most clinical settings. However, the WMC measures gastric emptying indirectly through the use of a physiologic meal. The pressure profiles are based on non-stationary, single point pressure measurements throughout the GI tract, which limits its utility in comparison to traditional manometric testing<sup>[45,46]</sup>.

## FUTURE DEVELOPMENTS

### *Trans-abdominal ultrasonography*

Ultrasonography represents a simple non-invasive technique to evaluate gastric function. Although operator dependent, ultrasonography provides information on gastric emptying, with a high correlation with scintigraphic techniques.

### *Magnetic resonance imaging*

Magnetic resonance techniques offer a potentially exciting non-invasive method for evaluating segmental and global motility within the GI tract, although protocols are currently limited to the research sphere. Nevertheless, given the widespread distribution of magnetic resonance imaging scanners across many clinical centres, it is likely that this method of imaging may become the method of choice in the future. However, further work is needed to standardize protocols and testing conditions.

## BIOCHEMICAL MARKERS OF NEUROINFLAMMATION IN DIABETES

Since the measurement of nerve conduction velocity *per se* is resource intensive, both in terms of equipment and specialist neurophysiological interpretation, the development and validation of non-invasive biomarkers remains an important priority. Considering the putative pathophysiological link between low-grade inflammation and diabetes<sup>[47]</sup>, we shall highlight some novel biochemical biomarkers, which have the potential to complement

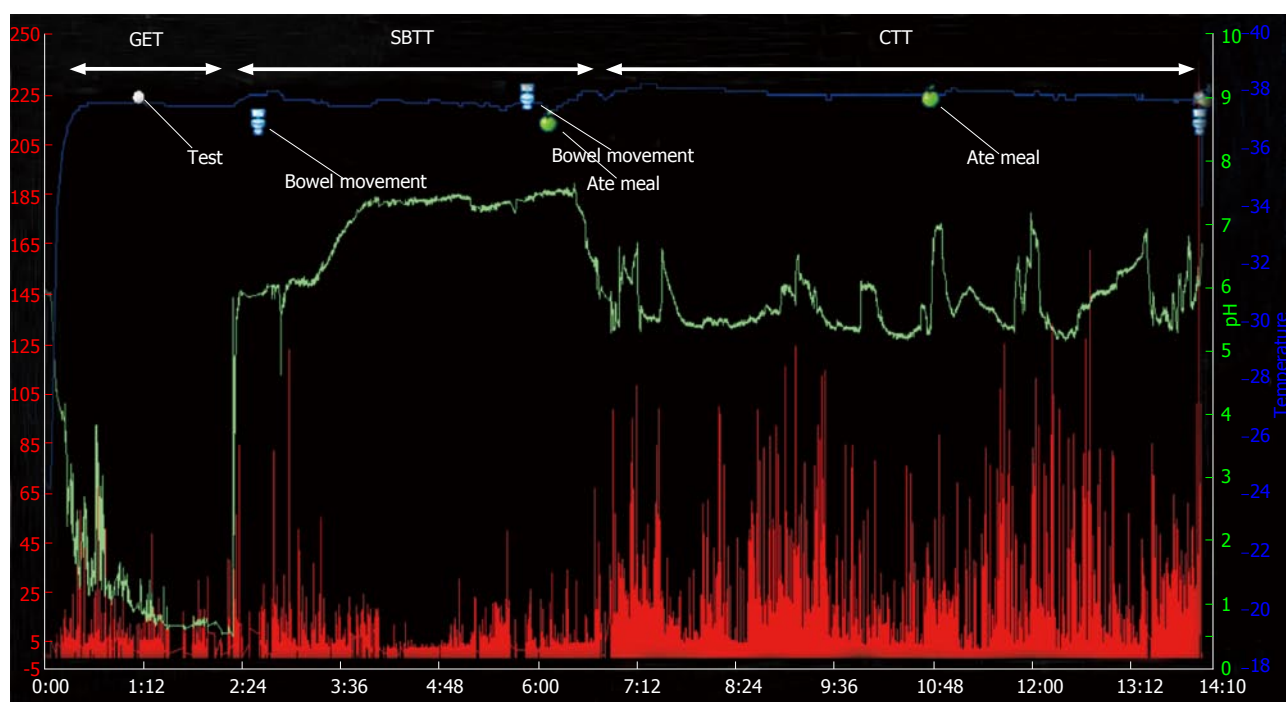


Figure 3 A typical tracing from a wireless motility capsule recording with time along the X-axis, pressure on the y1-axis (red line) and pH on the y2-axis (green line) and temperature (blue line). Gastric emptying time (GET), small bowel transit time (SBTT) and colonic transit time (CTT) are illustrated. Whole gut transit time is derived from the addition of GET, SBTT and CTT.

detailed neurophysiological testing in the future.

## ANTIBODIES TO NEURAL STRUCTURES

There is increasing evidence that the immune system may play a role in the genesis and maintenance of diabetic neuropathy. Therefore, autoantibodies directed towards neuronal structures have received considerable attention, although reports are conflicting. For instance, Zanone *et al.*<sup>[48]</sup> reported an association between autoantibodies directed against sympathetic ganglia, vagal afferents and the adrenal medulla in T1DM patients with symptomatic autonomic neuropathy.

In contrast however, Husebye *et al.*<sup>[49]</sup> did not demonstrate any quantitative differences in autoantibodies binding to adrenal medulla in T1DM or T2DM in comparison to health controls. Nevertheless, the objective demonstration of a causal pathway where the identification of antigens directed towards specific end organ neuronal targets, which can be reversed with neutralising antibodies, remains a prerequisite step.

## OXIDATIVE STRESS

Oxidative stress is considered a central facet in the development of diabetes and associated micro- and macrovascular complications. DNA and RNA oxidation have been linked to several diseases including diabetes. Whilst tissue specific levels of oxidation represent a single time point within a certain organ or cell system, urine excretion of 8-Oxo-2'-deoxyguanosine and 8-hydroxyguanine gives a more global measure of oxidative stress.

Not unsurprisingly therefore, it has been argued that in multi-system disorders, such as diabetes, such measures of global oxidative stress are of more pertinence. While an association between increased excretion of 8-Oxo-2'-deoxyguanosine in both diabetic retinopathy and nephropathy has been shown, there remains a paucity of data concerning in those patients with neuropathy. However, increased levels of 8-Oxo-2'-deoxyguanosine have been demonstrated in neurodegenerative disorders, such as Alzheimer's disease, and therefore such markers warrant further objective evaluation in patients with diabetic neuropathy.

## TUMOUR NECROSIS FACTOR-ALPHA

The influential cytokine theory of disease posits that a number of cytokines are involved in the maintenance of health and homeostasis within the peripheral, central and autonomic nervous systems. Cytokines are produced by cells from the immune system including mast cells, Schwann cells, fibroblasts and sensory neurons. Tumour necrosis factor-alpha (TNF- $\alpha$ ) is a potent systemic pro-inflammatory cytokine and is a central component of the inflammatory response, in various immune mediated inflammatory diseases. It is a pathophysiological feature of such disorders, such as rheumatoid arthritis, which are characterised by chronic inflammation. TNF- $\alpha$  is produced in Schwann cells and has a role in peripheral nerve regeneration and regulation of apoptosis. Elevated concentrations of TNF- $\alpha$  and heightened disease activity in immune mediated inflammatory disorders is well described, however, more recently a similar associa-

tion has been reported with neuropathy in diabetes T1DM and T2DM<sup>[49]</sup>. As such, TNF- $\alpha$  in diabetes may play a role in the pathogenesis and development of diabetic neuropathy and therefore could represent a candidate biomarker for the presence, severity and progression of diabetic neuropathy. Recent evidence provides further support for this proposition, as it has been showed that T2DM patients with neuropathy had higher levels of TNF- $\alpha$  in comparison to patients without neuropathy and healthy controls<sup>[50]</sup>. In addition, an animal model of painful diabetic neuropathy showed that treatment with anti-TNF- $\alpha$  monoclonal antibody exhibited a neuroprotective effect<sup>[51]</sup>. Finally, Yamakawa *et al.*<sup>[52]</sup> demonstrated that a single dose of anti-TNF- $\alpha$  attenuated the electrophysiological and biochemical deficits associated with diabetic neuropathy for at least 1 mo. To the best of our knowledge there are no reports in the literature of anti-TNF therapy being utilised in patients with established diabetic neuropathy, although it is plausible that it would benefit clinical symptoms in selected patient groups, although single case reports exists, which describes mixed sensorimotor neuropathies as a consequence of anti-TNF therapy.

## SOLUBLE CD163

CD163 is an endocytotic receptor for haptoglobin-haemoglobin complexes, which is expressed exclusively in macrophages and monocytes. The extracellular portion of CD163 is soluble (sCD163) and circulates in the peripheral blood. Although the absolute function of sCD163 is incompletely understood, an association is observed between increased circulating levels of and chronic inflammatory states<sup>[53]</sup>. Interestingly, increased levels of sCD163 have been reproducibly demonstrated in diabetes<sup>[54]</sup>. Furthermore, sCD163 has been shown to be associated with insulin resistance in T2DM; an association that is independent of TNF- $\alpha$ <sup>[55]</sup>. In addition, a trend towards increased levels of sCD163 has very recently been demonstrated in cerebrospinal fluid in a preliminary study of patients with T2DM with established diabetic polyneuropathy as compared to matched controls without neuropathy<sup>[56]</sup>. Taken together, these data provide an interesting rationale for the further evaluation in larger prospective studies of sCD163 as a candidate biomarker, particularly as it links both inflammatory and neuropathic processes.

Although there is a current paucity of non-invasive diagnostic and prognostic biomarkers for diabetic neuropathy, there are a number of promising candidates. Whilst singularly each has their respective limitations, in combination a higher clinical utility may be derived in the future.

## CONCLUSION

DAN remains an under-recognized complication, yet its symptomatic sequelae are troublesome and combine to reduce the quality of life and worsen prognosis in patients

with diabetes. Although biomarkers for early identification of DAN and testing for ANS dysfunction and its specific end-organ complications, such as in the GI tract, remain in their infancy, further objective evaluation is warranted to improve detection rates and diagnostic accuracy, which may potentially lead to improved patient outcomes.

## REFERENCES

- 1 Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract* 2010; **87**: 4-14 [PMID: 19896746 DOI: 10.1016/j.diabres.2009.10.007]
- 2 Narayan KM, Boyle JP, Geiss LS, Saaddine JB, Thompson TJ. Impact of recent increase in incidence on future diabetes burden: U.S., 2005-2050. *Diabetes Care* 2006; **29**: 2114-2116 [PMID: 16936162 DOI: 10.2337/dc06-1136]
- 3 FACTS and STATS Diabetes UK, november 2015. Available from: URL: <https://www.diabetes.org.uk/Documents>
- 4 Ewing DJ, Campbell IW, Clarke BF. The natural history of diabetic autonomic neuropathy. *Q J Med* 1980; **49**: 95-108 [PMID: 7433630]
- 5 Tesfaye S, Boulton AJ, Dyck PJ, Freeman R, Horowitz M, Kempler P, Lauria G, Malik RA, Spallone V, Vinik A, Bernardi L, Valensi P. Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. *Diabetes Care* 2010; **33**: 2285-2293 [PMID: 20876709 DOI: 10.2337/dc10-1303]
- 6 Abbott CA, Chaturvedi N, Malik RA, Salgami E, Yates AP, Pemberton PW, Boulton AJ. Explanations for the lower rates of diabetic neuropathy in Indian Asians versus Europeans. *Diabetes Care* 2010; **33**: 1325-1330 [PMID: 20215455 DOI: 10.2337/dc09-2067]
- 7 Østergaard L, Finnerup NB, Terkelsen AJ, Olesen RA, Drasbek KR, Knudsen L, Jespersen SN, Frystyk J, Charles M, Thomsen RW, Christiansen JS, Beck-Nielsen H, Jensen TS, Andersen H. The effects of capillary dysfunction on oxygen and glucose extraction in diabetic neuropathy. *Diabetologia* 2015; **58**: 666-677 [PMID: 25512003 DOI: 10.1007/s00125-014-3461-z]
- 8 Eaton SE, Harris ND, Ibrahim S, Patel KA, Selmi F, Radatz M, Ward JD, Tesfaye S. Increased sural nerve epineurial blood flow in human subjects with painful diabetic neuropathy. *Diabetologia* 2003; **46**: 934-939 [PMID: 12819899 DOI: 10.1007/s00125-003-1127-3]
- 9 Maser RE, Pfeifer MA, Dorman JS, Kuller LH, Becker DJ, Orchard TJ. Diabetic autonomic neuropathy and cardiovascular risk. Pittsburgh Epidemiology of Diabetes Complications Study III. *Arch Intern Med* 1990; **150**: 1218-1222 [PMID: 2353855]
- 10 Trotta D, Verrotti A, Salladini C, Chiarelli F. Diabetic neuropathy in children and adolescents. *Pediatr Diabetes* 2004; **5**: 44-57 [PMID: 15043690 DOI: 10.1111/j.1399-543x.2004.00041.x]
- 11 Verrotti A, Prezioso G, Scattoni R, Chiarelli F. Autonomic neuropathy in diabetes mellitus. *Front Endocrinol (Lausanne)* 2014; **5**: 205 [PMID: 25520703 DOI: 10.3389/fendo.2014.00205]
- 12 Sandireddy R, Yerra VG, Areti A, Komirishetty P, Kumar A. Neuroinflammation and oxidative stress in diabetic neuropathy: futuristic strategies based on these targets. *Int J Endocrinol* 2014; **2014**: 674987 [PMID: 24883061 DOI: 10.1155/2014/674987]
- 13 Vinik AI, Park TS, Stansberry KB, Pittenger GL. Diabetic neuropathies. *Diabetologia* 2000; **43**: 957-973 [PMID: 10990072 DOI: 10.1007/s001250051477]
- 14 Ekberg K, Johansson BL. Effect of C-peptide on diabetic neuropathy in patients with type 1 diabetes. *Exp Diabetes Res* 2008; **2008**: 457912 [PMID: 18350117 DOI: 10.1155/2008/457912]
- 15 Wahren J, Ekberg K, Johansson J, Henriksson M, Pramanik A, Johansson BL, Rigler R, Jörnvall H. Role of C-peptide in human physiology. *Am J Physiol Endocrinol Metab* 2000; **278**: E759-E768 [PMID: 10780930]
- 16 Krishnan AV, Kiernan MC. Altered nerve excitability properties in established diabetic neuropathy. *Brain* 2005; **128**: 1178-1187 [PMID: 15758031 DOI: 10.1093/brain/awh476]
- 17 Rudchenko A, Akude E, Cooper E. Synapses on sympathetic



- neurons and parasympathetic neurons differ in their vulnerability to diabetes. *J Neurosci* 2014; **34**: 8865-8874 [PMID: 24966386 DOI: 10.1523/JNEUROSCI.0033-14.2014]
- 18 **Kitada M**, Zhang Z, Mima A, King GL. Molecular mechanisms of diabetic vascular complications. *J Diabetes Investig* 2010; **1**: 77-89 [PMID: 24843412 DOI: 10.1111/j.2040-1124.2010.00018.x]
  - 19 **Williamson JR**, Chang K, Frangos M, Hasan KS, Ido Y, Kawamura T, Nyengaard JR, van den Enden M, Kilo C, Tilton RG. Hyperglycemic pseudohypoxia and diabetic complications. *Diabetes* 1993; **42**: 801-813 [PMID: 8495803 DOI: 10.2337/diab.42.6.801]
  - 20 **Chung SS**, Ho EC, Lam KS, Chung SK. Contribution of polyol pathway to diabetes-induced oxidative stress. *J Am Soc Nephrol* 2003; **14**: S233-S236 [PMID: 12874437]
  - 21 **Paravicini TM**, Touyz RM. NADPH oxidases, reactive oxygen species, and hypertension: clinical implications and therapeutic possibilities. *Diabetes Care* 2008; **31** Suppl 2: S170-S180 [PMID: 18227481 DOI: 10.2337/dc08-s247]
  - 22 **Harrison DG**, Widder J, Grumbach I, Chen W, Weber M, Searles C. Endothelial mechanotransduction, nitric oxide and vascular inflammation. *J Intern Med* 2006; **259**: 351-363 [PMID: 16594903 DOI: 10.1111/j.1365-2796.2006.01621.x]
  - 23 **Cameron NE**, Cotter MA, Jack AM, Basso MD, Hohman TC. Protein kinase C effects on nerve function, perfusion, Na(+), K(+)-ATPase activity and glutathione content in diabetic rats. *Diabetologia* 1999; **42**: 1120-1130 [PMID: 10447525 DOI: 10.1007/s001250051280]
  - 24 **Koya D**, King GL. Protein kinase C activation and the development of diabetic complications. *Diabetes* 1998; **47**: 859-866 [PMID: 9604860 DOI: 10.2337/diabetes.47.6.859]
  - 25 **Goldin A**, Beckman JA, Schmidt AM, Creager MA. Advanced glycation end products: sparking the development of diabetic vascular injury. *Circulation* 2006; **114**: 597-605 [PMID: 16894049 DOI: 10.1161/CIRCULATIONAHA.106.621854]
  - 26 **Flyvbjerg A**. Diabetic angiopathy, the complement system and the tumor necrosis factor superfamily. *Nat Rev Endocrinol* 2010; **6**: 94-101 [PMID: 20098449 DOI: 10.1038/nrendo.2009.266]
  - 27 **Lee ST**, Hon EH. The fetal electrocardiogram. iv. unusual variations in the QRS complex during labor. *Am J Obstet Gynecol* 1965; **92**: 1140-1148 [PMID: 14337038]
  - 28 **Brock C**, Søfteland E, Gunterberg V, Frøkjær JB, Lelic D, Brock B, Dimcevski G, Gregersen H, Simrén M, Drewes AM. Diabetic autonomic neuropathy affects symptom generation and brain-gut axis. *Diabetes Care* 2013; **36**: 3698-3705 [PMID: 24026548 DOI: 10.2337/dc13-0347]
  - 29 **Lelic D**, Brock C, Simrén M, Frøkjær JB, Søfteland E, Dimcevski G, Gregersen H, Drewes AM. The brain networks encoding visceral sensation in patients with gastrointestinal symptoms due to diabetic neuropathy. *Neurogastroenterol Motil* 2014; **26**: 46-58 [PMID: 24050116 DOI: 10.1111/nmo.12222]
  - 30 **Farmer AD**, Coen SJ, Kano M, Weltens N, Ly HG, Botha C, Paine PA, Oudenhove LV, Aziz Q. Normal values and reproducibility of the real-time index of vagal tone in healthy humans: a multi-center study. *Ann Gastroenterol* 2014; **27**: 362-368 [PMID: 25330916]
  - 31 **Tak LM**, Riese H, de Bock GH, Manoharan A, Kok IC, Rosmalen JG. As good as it gets? A meta-analysis and systematic review of methodological quality of heart rate variability studies in functional somatic disorders. *Biol Psychol* 2009; **82**: 101-110 [PMID: 19463887 DOI: 10.1016/j.biopsycho.2009.05.002]
  - 32 **Brock C**, Brock B, Pedersen AG. Novel Parameters of Parasympathetic Nervous System Tone-Noninvasive Biomarkers for Diabetic Autonomic Neuropathy? *Diabetes* 2015; **64**: 2441
  - 33 **Illigens BM**, Gibbons CH. Sweat testing to evaluate autonomic function. *Clin Auton Res* 2009; **19**: 79-87 [PMID: 18989618 DOI: 10.1007/s10286-008-0506-8]
  - 34 **Low VA**, Sandroni P, Fealey RD, Low PA. Detection of small-fiber neuropathy by sudomotor testing. *Muscle Nerve* 2006; **34**: 57-61 [PMID: 16718689 DOI: 10.1002/mus.20551]
  - 35 **Selvarajah D**, Cash T, Davies J, Sankar A, Rao G, Grieg M, Pallai S, Gandhi R, Wilkinson ID, Tesfaye S. SUDOSCAN: A Simple, Rapid, and Objective Method with Potential for Screening for Diabetic Peripheral Neuropathy. *PLoS One* 2015; **10**: e0138224 [PMID: 26457582 DOI: 10.1371/journal.pone.0138224]
  - 36 **Ponirakis G**, Petropoulos IN, Fadavi H, Alam U, Asghar O, Marshall A, Tavakoli M, Malik RA. The diagnostic accuracy of Neuropad for assessing large and small fibre diabetic neuropathy. *Diabet Med* 2014; **31**: 1673-1680 [PMID: 24975286 DOI: 10.1111/dme.12536]
  - 37 **Horváth VJ**, Izbéki F, Lengyel C, Kempler P, Várkonyi T. Diabetic gastroparesis: functional/morphologic background, diagnosis, and treatment options. *Curr Diab Rep* 2014; **14**: 527 [PMID: 25005121 DOI: 10.1007/s11892-014-0527-8]
  - 38 **Abell TL**, Camilleri M, Donohoe K, Hasler WL, Lin HC, Maurer AH, McCallum RW, Nowak T, Nusynowitz ML, Parkman HP, Shreve P, Szarka LA, Snape WJ, Ziessman HA. Consensus recommendations for gastric emptying scintigraphy: a joint report of the American Neurogastroenterology and Motility Society and the Society of Nuclear Medicine. *Am J Gastroenterol* 2008; **103**: 753-763 [PMID: 18028513 DOI: 10.1111/j.1572-0241.2007.01636.x]
  - 39 **Talley SJ**, Bytzer P, Hammer J, Young L, Jones M, Horowitz M. Psychological distress is linked to gastrointestinal symptoms in diabetes mellitus. *Am J Gastroenterol* 2001; **96**: 1033-1038 [PMID: 11316143 DOI: 10.1111/j.1572-0241.2001.03605.x]
  - 40 **Revicki DA**, Rentz AM, Tack J, Stanghellini V, Talley NJ, Kahrilas P, De La Loge C, Trudeau E, Dubois D. Responsiveness and interpretation of a symptom severity index specific to upper gastrointestinal disorders. *Clin Gastroenterol Hepatol* 2004; **2**: 769-777 [PMID: 15354277 DOI: 10.1016/S1542-3565(04)00348-9]
  - 41 **Chang CS**, Chen GH, Kao CH, Wang SJ, Peng SN, Poon SK, Huang CK. Gastric clearance of radiopaque markers in non-ulcer dyspepsia patients. *Scand J Gastroenterol* 1996; **31**: 136-139 [PMID: 8658035 DOI: 10.3109/00365529609031977]
  - 42 **Parkman HP**, Hasler WL, Fisher RS. American Gastroenterological Association technical review on the diagnosis and treatment of gastroparesis. *Gastroenterology* 2004; **127**: 1592-1622 [PMID: 15521026 DOI: 10.1053/j.gastro.2004.09.055]
  - 43 **Farmer AD**, Scott SM, Hobson AR. Gastrointestinal motility revisited: The wireless motility capsule. *United European Gastroenterol J* 2013; **1**: 413-421 [PMID: 24917991 DOI: 10.1177/2050640613510161]
  - 44 **Lee YY**, Erdogan A, Rao SS. How to assess regional and whole gut transit time with wireless motility capsule. *J Neurogastroenterol Motil* 2014; **20**: 265-270 [PMID: 24840380 DOI: 10.5056/jnm.2014.20.2.265]
  - 45 **Tran K**, Brun R, Kuo B. Evaluation of regional and whole gut motility using the wireless motility capsule: relevance in clinical practice. *Therap Adv Gastroenterol* 2012; **5**: 249-260 [PMID: 22778790 DOI: 10.1177/1756283x12437874]
  - 46 **Cassilly D**, Kantor S, Knight LC, Maurer AH, Fisher RS, Semler J, Parkman HP. Gastric emptying of a non-digestible solid: assessment with simultaneous SmartPill pH and pressure capsule, antroduodenal manometry, gastric emptying scintigraphy. *Neurogastroenterol Motil* 2008; **20**: 311-319 [PMID: 18194154 DOI: 10.1111/j.1365-2982.2007.01061.x]
  - 47 **Duncan BB**, Schmidt MI, Pankow JS, Ballantyne CM, Couper D, Vigo A, Hoogeveen R, Folsom AR, Heiss G. Low-grade systemic inflammation and the development of type 2 diabetes: the atherosclerosis risk in communities study. *Diabetes* 2003; **52**: 1799-1805 [PMID: 12829649]
  - 48 **Zanone MM**, Peakman M, Purewal T, Watkins PJ, Vergani D. Autoantibodies to nervous tissue structures are associated with autonomic neuropathy in type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 1993; **36**: 564-569 [PMID: 8335180 DOI: 10.1007/BF02743275]
  - 49 **Husebye ES**, Winqvist O, Sundkvist G, Kämpe O, Karlsson FA. Autoantibodies against adrenal medulla in type 1 and type 2 diabetes mellitus: no evidence for an association with autonomic neuropathy. *J Intern Med* 1996; **239**: 139-146 [PMID: 8568481 DOI: 10.1046/j.1365-2796.1996.423766000.x]
  - 50 **Zhu T**, Meng Q, Ji J, Lou X, Zhang L. Toll-like receptor 4 and tumor necrosis factor-alpha as diagnostic biomarkers for diabetic



- peripheral neuropathy. *Neurosci Lett* 2015; **585**: 28-32 [PMID: 25445373 DOI: 10.1016/j.neulet.2014.11.020]
- 51 **Dogrul A**, Gul H, Yesilyurt O, Ulas UH, Yildiz O. Systemic and spinal administration of etanercept, a tumor necrosis factor alpha inhibitor, blocks tactile allodynia in diabetic mice. *Acta Diabetol* 2011; **48**: 135-142 [PMID: 21104419 DOI: 10.1007/s00592-010-0237-x]
  - 52 **Yamakawa I**, Kojima H, Terashima T, Katagi M, Oi J, Urabe H, Sanada M, Kawai H, Chan L, Yasuda H, Maegawa H, Kimura H. Inactivation of TNF- $\alpha$  ameliorates diabetic neuropathy in mice. *Am J Physiol Endocrinol Metab* 2011; **301**: E844-E852 [PMID: 21810933 DOI: 10.1152/ajpendo.00029.2011]
  - 53 **Møller HJ**. Soluble CD163. *Scand J Clin Lab Invest* 2012; **72**: 1-13 [PMID: 22060747 DOI: 10.3109/00365513.2011.626868]
  - 54 **Etzerodt A**, Moestrup SK. CD163 and inflammation: biological, diagnostic, and therapeutic aspects. *Antioxid Redox Signal* 2013; **18**: 2352-2363 [PMID: 22900885 DOI: 10.1089/ars.2012.4834]
  - 55 **Llauradó G**, González-Clemente JM, Maymó-Masip E, Subías D, Vendrell J, Chacón MR. Serum levels of TWEAK and scavenger receptor CD163 in type 1 diabetes mellitus: relationship with cardiovascular risk factors. a case-control study. *PLoS One* 2012; **7**: e43919 [PMID: 22937125 DOI: 10.1371/journal.pone.0043919]
  - 56 **Parkner T**, Sørensen LP, Nielsen AR, Fischer CP, Bibby BM, Nielsen S, Pedersen BK, Møller HJ. Soluble CD163: a biomarker linking macrophages and insulin resistance. *Diabetologia* 2012; **55**: 1856-1862 [PMID: 22450890 DOI: 10.1007/s00125-012-2533-1]
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## Treatment of diabetic retinopathy: Recent advances and unresolved challenges

Michael W Stewart

Michael W Stewart, Department of Ophthalmology, Mayo Clinic Florida, Jacksonville, FL 32224, United States

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**Correspondence to:** Michael W Stewart, MD, Department of Ophthalmology, Mayo Clinic Florida, 4500 San Pablo Rd., Jacksonville, FL 32224, United States. [stewart.michael@mayo.edu](mailto:stewart.michael@mayo.edu)  
 Telephone: +1-904-9532232  
 Fax: +1-904-9537040

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### Abstract

Diabetic retinopathy (DR) is the leading cause of blindness in industrialized countries. Remarkable advances in the diagnosis and treatment of DR have been made during the past 30 years, but several important management

questions and treatment deficiencies remain unanswered. The global diabetes epidemic threatens to overwhelm resources and increase the incidence of blindness, necessitating the development of innovative programs to diagnose and treat patients. The introduction and rapid adoption of intravitreal pharmacologic agents, particularly drugs that block the actions of vascular endothelial growth factor (VEGF) and corticosteroids, have changed the goal of DR treatment from stabilization of vision to improvement. Anti-VEGF injections improve visual acuity in patients with diabetic macular edema (DME) from 8-12 letters and improvements with corticosteroids are only slightly less. Unfortunately, a third of patients have an incomplete response to anti-VEGF therapy, but the best second-line therapy remains unknown. Current first-line therapy requires monthly visits and injections; longer acting therapies are needed to free up healthcare resources and improve patient compliance. VEGF suppression may be as effective as panretinal photocoagulation (PRP) for proliferative diabetic retinopathy, but more studies are needed before PRP is abandoned. For over 30 years laser was the mainstay for the treatment of DME, but recent studies question its role in the pharmacologic era. Aggressive treatment improves vision in most patients, but many still do not achieve reading and driving vision. New drugs are needed to add to gains achieved with available therapies.

**Key words:** Aflibercept; Bevacizumab; Dexamethasone delivery system; Diabetic macular edema; Ranibizumab; Macular photocoagulation; Panretinal photocoagulation; Proliferative diabetic retinopathy; Diabetic retinopathy; Fluocinolone acetonide insert

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**Core tip:** Newly introduced pharmacotherapies have contributed significantly to the treatment of diabetic retinopathy over the past 10 years and have become first-line therapy. Several questions regarding the best management of certain diabetic conditions remain and

new therapies are needed to improve outcomes. Ongoing research and development should address many of these issues over the next 10 years.

Stewart MW. Treatment of diabetic retinopathy: Recent advances and unresolved challenges. *World J Diabetes* 2016; 7(16): 333-341 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v7/i16/333.htm> DOI: <http://dx.doi.org/10.4239/wjd.v7.i16.333>

## INTRODUCTION

Diabetes mellitus (DM) has become a global health problem fueled by increased caloric consumption and the resultant obesity epidemic<sup>[1]</sup>. Microvascular complications of DM (retinopathy, nephropathy and neuropathy) are increasingly important causes of morbidity and mortality, and care for affected patients contributes to burgeoning healthcare costs. Diabetic retinopathy (DR) is the leading cause of blindness in working-aged individuals in industrialized nations<sup>[2]</sup>; diabetic macular edema (DME) accounts for 75% of DR-related vision loss with complications of proliferative diabetic retinopathy (PDR) responsible for most of the balance.

The development, testing, and adoption of advanced intravitreal pharmacotherapy has significantly improved the treatment of diabetic retinopathy over the past decade. The pivotal phase III drug trials demonstrated that binding diffusible vascular endothelial growth factor (VEGF) improves visual acuity (VA) in the majority of patients. Monthly injections of ranibizumab (Lucentis®, Genentech, S. San Francisco, CA, United States/Roche, Basel, Switzerland) and aflibercept (Eylea®, Regeneron, Tarrytown, NY, United States) prevent progression to PDR in many high risk eyes and reverse diabetic retinopathy severity scores in a significant minority<sup>[3,4]</sup>. Ranibizumab injections are as effective as panretinal photocoagulation at controlling the complications of PDR, while causing fewer visual side effects<sup>[5]</sup>.

These tremendous advances could not have been imagined by most practitioners 20 years ago, but many questions regarding the optimal therapy for different clinical situations remain unanswered (Figure 1). Additionally, the majority of the world's population does not have access to specialized care and affordable drugs. This manuscript will discuss some of the unmet challenges pertaining to the treatment of diabetic retinopathy care and will speculate on anticipated solutions.

## HOW WILL WE CARE FOR THE INCREASING NUMBER OF PATIENTS WITH DIABETIC RETINOPATHY?

Effective management of DR depends heavily on early diagnosis and prompt treatment. Laser photocoagulation of DME according to the Early Treatment of Diabetic Retinopathy Study (ETDRS) guidelines for clinically signi-

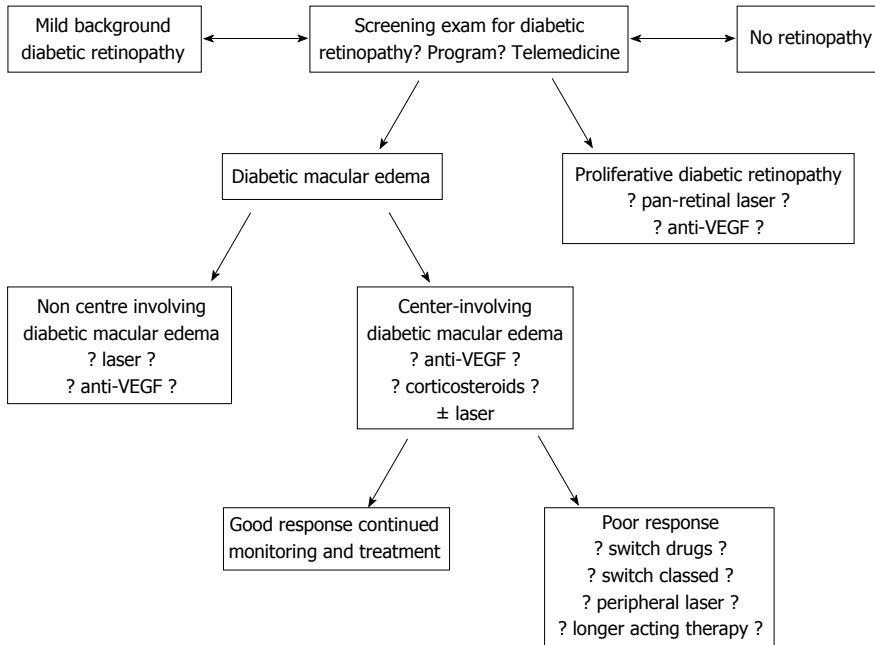
ficant macular edema decreases the risk of moderate vision loss (15 letters) by 50% over three years, but far fewer patients experience comparable VA gains<sup>[6]</sup>. Anti-VEGF therapy, on the other hand, improves VA by a mean of 8-12 ETDRS letters, but eyes with significant acuity loss (< 20/100) usually do not recover reading or driving vision<sup>[3,4]</sup>.

Early diagnoses of DR are made by dilated fundus examinations that are usually performed after patients are referred to ophthalmologists or are seen within a DR screening program. Prominent professional organizations including the American Academy of Pediatrics, the American Academy of Ophthalmology, the American Diabetes Association, and the Canadian Ophthalmological Society have published screening guidelines<sup>[7-10]</sup>. These generally agree that patients need yearly dilated fundus examinations beginning at the time of diagnosis for patients with type 2 DM and after an interval of 3-5 years for patients with type 1 DM. Despite long-standing efforts to educate patients, primary care physicians, and endocrinologists, only 50% of patients undergo screening eye examinations in any given year and 16% receive exams in two consecutive years<sup>[11]</sup>.

Many industrialized countries have sufficient ophthalmologists to effectively screen their diabetic populations, but non-compliance with screening guidelines is common because of socioeconomic, cultural, and geographic reasons<sup>[12]</sup>. Patients may be unaware of diabetes-related risks of vision loss and primary care physicians' referral patterns may be inadequate. Education programs directed at both physicians and patients need improvement, but these are unlikely to completely fix deficiencies in screening<sup>[13]</sup>.

Screening programs are particularly important in developing countries where an insufficient number of physicians and long travel distances preclude the performance of recommended dilated fundus examinations<sup>[14]</sup>. Telemedicine has the potential to deliver affordable care to many of these underserved populations. Telemedicine DR screening programs can be created with modest up-front purchases of standard or non-mydiatic fundus cameras combined with training of office personnel to take high-quality fundus photographs. Newly developed cellular telephone adapters can turn nearly any telephone into a high-quality fundus camera<sup>[15]</sup>. The low cost of the adapters together with the widespread availability of cellular telephones allows for placement of a fundus camera in nearly any office. Patients can then receive high quality retinopathy screening evaluations in their primary physicians' offices. Photos are electronically transmitted to ophthalmologists' offices or image reading centers for evaluation. Patients with high-risk fundus abnormalities can be referred for ophthalmology examinations or scheduled for future photographs. Issues concerning Health Insurance Portability and Accountability Act compliance and insurance billing can be challenging, but systems that successfully address these concerns have been developed.

Photographs are transmitted *via* the internet to reading centers staffed with readers. Some centers employ



**Figure 1** This drawing identifies many of the unresolved treatment questions pertaining to the evaluation and treatment of patients with diabetic retinopathy. The question marks highlight areas of controversy that are discussed in greater detail in the manuscript. VEGF: Vascular endothelial growth factor.

specially trained technicians to read submitted clinical photographs, whereas others use ophthalmologists and retina specialists. As screening programs expand and reach more patients the number of transmitted photographs may overwhelm the ability of reading centers to properly evaluate them. Computer programs are being developed to digitally identify abnormalities on photos, make a diagnosis, assess the risk of vision loss, and recommend referral to an ophthalmologist or defer for future screening<sup>[16,17]</sup>. Developing and validating software is a complicated task, and powerful hardware is needed to read thousands of photographs, but software developers in several countries are developing programs that may be commercially available within 5 years.

## WHAT IS THE BEST APPROACH TO TREATMENT-NAÏVE DME?

The pivotal phase III anti-VEGF trials evaluated the efficacy of monthly (ranibizumab and aflibercept) or bimonthly (aflibercept) injections on center-involving DME<sup>[3,4]</sup>. Patients switched to pro re nata (PRN) ranibizumab after 12 mo in RESTORE<sup>[18]</sup> and after 36 mo in RISE/RIDE<sup>[19]</sup>. Treating patients according to these strategies probably produces the best possible visual results, but treatment is expensive and compliance is difficult to maintain. Diabetic Retinopathy Clinical Research Network (DRCR.net) Protocols I<sup>[20]</sup> and T<sup>[21]</sup> used monthly injections for 4 mo or until dry before switching to monthly PRN protocols based on retreatment criteria that many physicians believe are too complex to use in most clinical settings. Neovascular age-related macular degeneration (nAMD) may be adequately treated with monthly PRN injections after single loading doses<sup>[22]</sup>, but most investigators be-

lieve that DME responds slower to therapy and that a prolonged series of initial injections is needed. Monthly treatment regimens, however, conflict with the real-world treatment of DME as most physicians use a treat-and-extend strategy (T and E) regimen for both nAMD and DM<sup>[23]</sup>.

As-needed (PRN) treatment regimens reduce the number of injections, but not the number of clinic visits. The 24-mo, single-masked RETAIN trial compared T and E + laser, T and E, and PRN ranibizumab regimens in patients with DME<sup>[24]</sup>. Patients in all groups were treated monthly until dry. The VA improvement in patients receiving T and E + laser, T and E, and PRN were similar (+5.9, +6.1, +6.2 letters). The mean numbers of injections were 12.4, 12.8, and 10.7, but patients treated with T and E required 46% fewer clinic visits. Over 70% of patients had treatment intervals extended to at least 2 mo. Though trial design differences make it difficult to directly compare these data to those from the phase III trials, the results are encouraging and will not deter physicians from employing a T and E regimen. A multicenter, randomized trial comparing monthly therapy with T and E is needed, but its cost would likely be prohibitive.

The National Eye Institute funded DRCR.net Protocol T gave us the best comparative data for the three anti-VEGF drugs<sup>[21]</sup>. For eyes with baseline visual acuities of 20/32–20/40, each of the drugs produced VA gains of +8 letters. But for eyes with baseline VA of 20/50 or worse, aflibercept (+18.9 letters) produced greater gains in VA than ranibizumab (+14.2 letters) and bevacizumab (+11.8 letters). Aflibercept also produced greater macular thinning (–169  $\mu$ m) than either ranibizumab (–147  $\mu$ m) or bevacizumab (–101  $\mu$ m). On average, patients received fewer aflibercept (9) than ranibizumab (10) or bevacizumab (10) injections.



Several issues (compounding of bevacizumab, use of ETDRS visual acuity, complex retreatment criteria) call into question the applicability of the Protocol T data. But many physicians use the results literally; for patients with VA of 20/40 or better they use bevacizumab because of its lower cost; for eyes with VA of 20/50 or worse they use aflibercept because of its greater efficacy<sup>[25]</sup>. Additional trials are needed to validate these data and identify additional subgroups that respond particularly well or poorly to treatment.

## ARE CORTICOSTEROIDS AS EFFECTIVE AS ANTI-VEGF DRUGS?

The dexamethasone delivery system (DDS, Ozurdex<sup>®</sup>, Allergan, Irvine, CA, United States) and the fluocinolone acetonide insert (Iluvien<sup>®</sup>, Alimera Sciences, Alpharetta, GA, United States) have both been approved for the treatment of DME. The DDS was originally approved for use in pseudophakic eyes or phakic eyes scheduled to undergo cataract removal, but approval for use in phakic eyes followed within months. Unfortunately, neither drug has been directly compared to anti-VEGF therapy in prospective, masked, randomized, multicenter trials. Visual acuity improvements for these sustained delivery systems average +7 letters<sup>[26,27]</sup>, generally less than the +8 to +12 letters achieved with anti-VEGF therapy. Patients in the FAME trial (fluocinolone insert) with DME of > 3 years duration responded better to the insert than did those with non-chronic DME. This suggests that chronic DME is chemokine-driven whereas non-chronic DME is VEGF driven<sup>[28]</sup>. Perhaps the best treatment strategy is to use anti-VEGF drugs for non-chronic DME and reserve corticosteroid use for chronic DME. Three years of corticosteroid therapy leads to high rates of cataract development (91%) and elevated intraocular pressure (> 30%). For these reasons, intraocular corticosteroids may be effective second-line therapy, but are usually not used as first-line therapy.

Small, single center studies suggest that the DDS produces comparable VA improvements to those of bevacizumab and ranibizumab, and perhaps superior macular thinning, but randomized, multicenter trials are needed to determine relative efficacies.

## CAN WE DEVELOP LONGER ACTING THERAPIES?

Monthly anti-VEGF therapy appears to decrease the need for therapy after one year<sup>[29]</sup> and improves DR severity after two years<sup>[3]</sup>. By contrast, nAMD appears to require therapy for longer periods of time<sup>[30]</sup>. Not surprisingly, development of anti-VEGF drugs has been driven primarily by the need for nAMD therapy. Phase III nAMD trials using longer acting anti-VEGF agents such as abicipar pegol, a designed ankyrin repeat protein, and RTH508, a single-strand antibody fragment, are underway. A phase I / II multicenter, dose-escalation trial evaluated the safety

and bioactivity of abicipar in 18 patients with DME<sup>[31]</sup>. Patients in the 1 mg cohort who received single injections experienced excellent reduction in macular thickness and average VA improvements of +10 letters at 12 wk. Pharmacokinetic analyses based on anterior chamber drug concentrations suggested an extended intraocular half-life of 13.4 d. The phase III nAMD trial is hoping to show efficacy with q12week dosing that is comparable to q4week ranibizumab<sup>[32]</sup>. A phase II DME trial has not yet been announced.

A refillable, trans-scleral ranibizumab reservoir underwent phase I nAMD testing with 20 patients in Latvia<sup>[33]</sup>. Patients required an average of 4.8 refills of the reservoir (500 µg each time) and achieved VA improvements comparable to those from the pivotal phase III trials. Four of the 20 eyes experienced significant complications due to implantation, requiring a modification of the surgical technique.

An anti-VEGF producing encapsulated cell chamber filled with immortalized retinal pigment epithelial cells is entering phase II trials for nAMD<sup>[34]</sup>. The genetically modified cells produce a high-affinity, VEGF-binding protein. In a previous trial this technology produced ciliary neurotrophic factor with a cell half-life of 51 mo<sup>[35]</sup> and developers are hoping that the current reservoir successfully treats nAMD for at least 24 mo. Since cells in the encapsulated chamber can be genetically modified to produce any molecule, the production of future DME drugs and even combination therapy may be possible. Should nAMD trials prove successful then testing for DME and DR will undoubtedly follow.

## WHEN SHOULD WE INITIATE PHARMACOLOGIC THERAPY FOR DR?

The ETDRS study focused on preventing vision loss so it enrolled patients with clinically significant macular edema (center-involving or center-threatening edema) and VA as good as 20/20<sup>[6]</sup>. Laser photocoagulation decreased the 3-year incidence of moderate vision loss (15 letters) by 50%, but is accompanied by the risk of laser-induced paracentral scotomas. In contrast, the anti-VEGF trials had upper VA limits of 20/32 or 20/40. Therefore, we have no good data from the anti-VEGF era regarding the best treatment for eyes with DME and VA better than 20/32.

Most physicians agree that laser photocoagulation is an excellent treatment for center-threatening DME with good VA. Laser produces a durable effect with few complications, but the best approach to center-involving DME and VA better than 20/32 is not known. Some physicians will treat symptomatic patients with center-involving DME and excellent VA with anti-VEGF drugs despite the off-label indication. The DRCR.net Protocol V is comparing laser photocoagulation with intravitreal aflibercept for this population<sup>[36]</sup>. The study's primary goal is to evaluate the effect of therapy on VA and DME, but the chance of decreasing the DR severity score makes this a very interesting study.

## WHAT IS THE BEST TREATMENT FOR PROLIFERATIVE DIABETIC RETINOPATHY?

The DRS showed that timely pan-retinal photocoagulation of high-risk PDR decreases the risk of severe vision loss by over 50%<sup>[37]</sup>. Laser photocoagulation obliterates mid-peripheral areas of ischemic retina, thereby down-regulating VEGF synthesis and promoting regression of neovascularization (NV). Unfortunately, broad areas of photocoagulation lead to permanently decreased peripheral vision and impaired night vision.

Anti-VEGF drugs involute optic disk neovascularization<sup>[38,39]</sup>, but the effect of a single intravitreal injection is transient as retinal NV recurs by 12 wk<sup>[40]</sup>. Pre-operative and intra-operative injections of bevacizumab facilitate fibrovascular membrane dissection, and reduce intra-operative and post-operative bleeding. Anti-VEGFs have been used to prevent additional bleeding from PDR while waiting for vitreous hemorrhage to clear<sup>[41]</sup> though the long-term visual benefits with this approach are questionable.

The DRCR.net performed a multi-center (55 sites), randomized clinical trial comparing panretinal photocoagulation with intravitreal 0.5 mg ranibizumab in 305 patients with PDR<sup>[5]</sup>. PRP was performed at baseline and ranibizumab was given at baseline and q4week PRN. Eyes with DME in both groups were eligible to receive ranibizumab. The primary outcome was change in best corrected visual acuity (BCVA) and the secondary outcomes included area under the VA curve, peripheral visual field loss (as measured on Humphrey automated visual field testing), incidence of vitrectomy, development of DME, and persistent or new neovascularization. Improvements in BCVA for the ranibizumab and PRP groups were +2.2 and +0.2 letters respectively (95%CI: -0.5 to +5.0). The group receiving ranibizumab experienced less peripheral visual field sensitivity loss (-23 dB vs -422 dB; 95%CI: 213-531 dB;  $P < 0.001$ ), fewer vitrectomies (4% vs 15%; 95%CI: 4%-15%;  $P < 0.001$ ), and a lower incidence of DME (9% vs 28%). Ranibizumab treated eyes required a median of 7 injections through year 1 and 10 injections through year 2. Forty-five percent of eyes in the PRP group required additional laser and 53% of eyes required ranibizumab for DME. The authors concluded that ranibizumab may be a reasonable alternative to PRP through 2 years. The decreasing number of injections in year 2 suggests that some disease modulation occurs after 1 year of ranibizumab therapy.

These data are likely to promote a paradigm shift in the treatment of PDR. Compared to PRP, ranibizumab involutes NV while significantly preserving visual function. Ranibizumab may be a favorable alternative to PRP for compliant patients. Some physicians who prefer anti-VEGF therapy over laser will probably use bevacizumab and aflibercept, though data for these drugs are not yet available. Confirmatory studies with ranibizumab are

needed as well as similarly structured trials with bevacizumab and aflibercept.

## WHAT IS THE ROLE OF LASER PHOTOCOAGULATION IN THE TREATMENT OF DME?

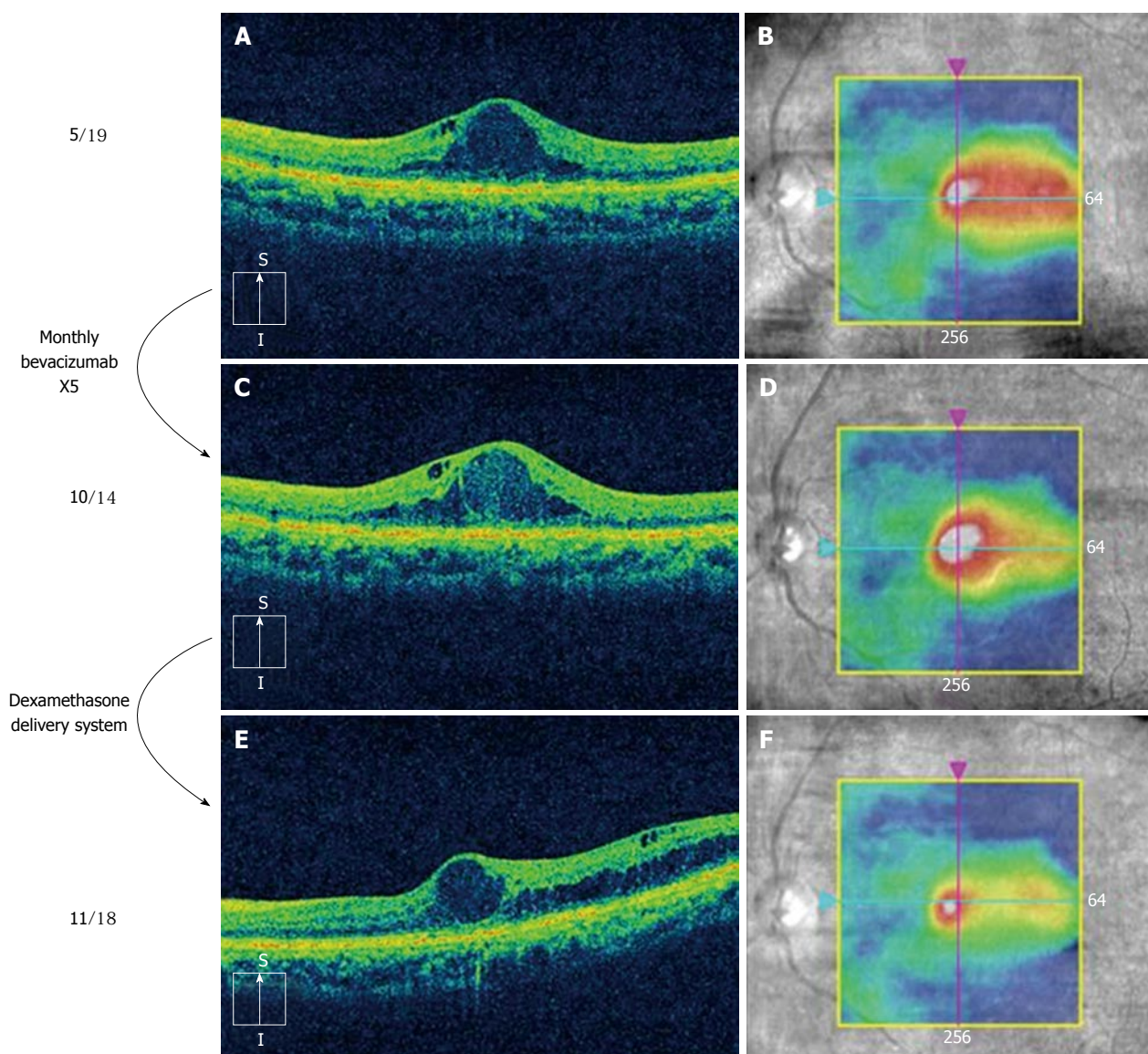
Anti-VEGF therapy has replaced macular laser photocoagulation for center-involving DME, but the anti-VEGF arms in all of the major anti-VEGF trials used laser as a rescue therapy. DRCR.net Protocol I combined ranibizumab with immediate or deferred (for at least 6 mo) laser<sup>[20]</sup>; Protocol T allowed laser after 6 mo<sup>[21]</sup>; RISE and RIDE allowed laser after 3 mo<sup>[3]</sup>; and VIVID/VISTA allowed laser after 3 mo<sup>[4]</sup>.

RESTORE was the only trial to test 1 year of anti-VEGF monotherapy and these eyes experienced better visual improvement than the ranibizumab + laser arm<sup>[18]</sup>. Eyes in the ranibizumab + deferred laser arm of Protocol I achieved better visual improvement than those in the ranibizumab + prompt laser arm<sup>[20]</sup>. These data suggest that macular laser photocoagulation may actually limit visual acuity in eyes with DME. Some investigators perform early laser photocoagulation to decrease the number of necessary anti-VEGF injections, but Protocol I showed that patients in the prompt laser arm required 3 laser treatments in order to reduce the number of ranibizumab injections by 3<sup>[29]</sup>. Macular laser, therefore, appears to add little durability to a ranibizumab regimen.

A post hoc subset analysis of the RESTORE data showed that laser photocoagulation improved VA as well as monthly ranibizumab in eyes with central retinal thickness (CRT)  $< 400 \mu\text{m}$ <sup>[42]</sup>. This led the National Institute for Health Care Excellence (United Kingdom) to approve ranibizumab only for eyes with CRT  $> 400 \mu\text{m}$ . Comparable findings from RISE/RIDE and VIVID/VISTA have not been reported so the RESTORE data are not generalizable. Additional analyses of treatment effects according to baseline parameters such as CRT, ellipsoid zone integrity, and inner retinal edema need to be done.

Some physicians use the micropulse laser to treat macular edema due to DR and branch retinal vein occlusions. The laser delivers low-energy, millisecond bursts of laser to the photoreceptors and retinal pigment epithelium without causing permanent damage. Unlike standard photocoagulation, treatment to the same areas can be repeated. Small, retrospective studies have shown improvements in macular edema and visual acuity<sup>[43,44]</sup>, but large, randomized, controlled trials against standard laser photocoagulation and in combination with anti-VEGF therapy need to be done.

Fluorescein angiographic studies with ultra-wide field (200°) cameras reveal large areas of peripheral retinal capillary non-perfusion in eyes with DME. DME recurs frequently in these eyes despite frequent anti-VEGF injections. Many investigators believe that these areas synthesize a continuous stream of VEGF that perpetuates



**Figure 2** This figure shows sequential optical coherence tomography scans of a patient with diabetic macular edema. At baseline (A and B) the patient was given a series of 5 monthly injections of bevacizumab with no significant improvement in macular edema (C and D); one month after a single injection of the dexamethasone delivery system, the volume of edema had significantly improved (E and F).

the macular edema. Obliteration of these areas with scatter laser photocoagulation has produced inconsistent improvements in DME<sup>[45]</sup>. Randomized, controlled studies of image-guided peripheral laser photocoagulation are needed to assess the feasibility of this strategy.

## WHAT IS THE BEST TREATMENT FOR POOR RESPONDERS?

Anti-VEGF therapy incompletely resolves edema in 20% to 40% of eyes and in the pivotal trials, laser photocoagulation after 3 to 6 mo was the only rescue therapy available for incomplete responders. Since macular laser photocoagulation has not been shown to improve VA, the best approach to incomplete responders is not known.

Now that ranibizumab and aflibercept have been Food and Drug Administration (FDA) approved for the

treatment of DME, switching from a lower to a higher VEGF binding affinity drug (VEGF binding affinities: Bevacizumab < ranibizumab < aflibercept) has been studied<sup>[46]</sup>. This approach appears to improve macular thickening and modestly improve VA, but randomized, controlled studies need to be performed.

A popular approach to the treatment of incomplete responders is to switch from an anti-VEGF to a corticosteroid (usually DDS) (Figure 2) or add a corticosteroid (combination therapy). This frequently improves macular thickness, but additional VA gains are variable<sup>[47]</sup>. The fluocinolone acetonide (FA) insert works best in eyes with chronic DME, presumably because long-standing edema (> 3 years) is primarily driven by chemokines and not VEGF. However, the FA insert has not been studied in eyes that respond incompletely to anti-VEGF therapy so its efficacy in incomplete responders is unknown.

Pars plana vitrectomy has been performed in eyes



**Table 1** The drugs under current development for the treatment of diabetic macular edema

Drug	Study design	Results
Danazol	23 eyes: 12-wk placebo-control	Significant decreases in CRT (-86% vs -29%) and macular volume ( $P = 0.05$ )
Minocycline	5 eyes: Minocycline 100 mg BID for 6 mo	Improvement in BCVA by 1 category (14% vs 47%) Improved BCVA and CRT compared to historic controls
Loteprednol etabonate (topical)	20 eyes: Single masked, 2-dose, randomized trial	Phase II clinical trial (KPI-121-C-004) underway
Dexamethasone phosphate	Iontophoresis driven into the eye EGP-437	Positive results in 15 patients
PAN-90806	4 monotherapy arms in phase I / II trial	Maintenance therapy after single anti-VEGF injections
Diclofenac	57 eyes: Intravitreal diclofenac vs bevacizumab	Diclofenac achieved better improvement in BCVA compared to bevacizumab ( $\Delta -0.08$ LogMAR vs $\Delta +0.04$ LogMAR, $P = 0.033$ ) Bevacizumab improved macular edema slightly better
Sirolimus	Phase I trial: Dose-escalating, subconjunctival of intravitreal injections	Subconjunctival: median increase in BCVA was +4.0 letters at 45 d
Fasudil	Single intravitreal injections of fasudil with bevacizumab	Intravitreal: Median increase in BCVA was +4.0 letters at 90 d At 4 wk $\Delta$ BCVA ( $0.84 \pm 0.35$ LogMAR to $0.49 \pm 0.29$ LogMAR; $P = 0.003$ ) and mean $\Delta$ CRT ( $448 \pm 123 \mu\text{m}$ to $347 \pm 76 \mu\text{m}$ ; $P = 0.001$ )
Luminate (ALG-001)	Phase II b DME trial (targets integrin receptors)	Data expected third quarter of 2016
Plasma kallikrein inhibitor (KVD001)	Phase II trials: Monotherapy for resistant DME combined with anti-VEGF	Phase I trial demonstrated safety after intravitreal injections
REGN910 (Ang 2 Ab)	Phase I trial	Completed. Now planning phase II trials for nAMD and DME

Important drug characteristics and early phase trial results are documented. CRT: Central retina thickness; BCVA: Best corrected visual acuity; VEGF: Vascular endothelial growth factor; LogMAR: Logarithm of minimal angle of resolution; DME: Diabetic macular edema; Ang: Angiotensin; Ab: Antibody; nAMD: Neovascular age-related macular degeneration; BID: Twice daily; EGP: Eyegate pharmaceuticals.

with DME for over 20 years and it appears to be more commonly used in Europe and Japan than in the United States. Vitrectomy was originally studied in eyes with biomicroscopically visible vitreomacular traction<sup>[48]</sup>, but its use has been expanded to eyes both with and without optical coherence tomography (OCT) identified traction. Many studies have reported excellent results<sup>[49,50]</sup>, but a meta-analysis that included only 11 studies showed no significant response to surgery<sup>[51]</sup>. A DRCR.net study of eyes that had failed previous therapy showed that vitrectomy improves macular edema, but not mean VA<sup>[52]</sup>. The high variability in VA responses indicated that eyes did either very well or very poorly and the study was not able to predict responses based on baseline findings. Recent studies with pre-operative spectral domain OCT analyses of outer retina integrity (external limiting membrane and ellipsoid zone) suggest that vitrectomy holds promise as an early treatment for DME, but this remains to be proven<sup>[53]</sup>.

## CAN WE DEVELOP MORE POTENT PHARMACOLOGIC AGENTS?

We are fortunate to have a robust development pipeline for new DME drugs. Some of these drugs have already been mentioned in this manuscript, and others are listed in the Table 1. Most of these drugs are in phase I and II testing so clinical availability would not be expected for 5 to 10 years. Their potential uses vary from disease modulation in patients with early DR, to monotherapy or combination therapy for patients with established DME.

Many of these drugs will not receive FDA approval, but others will provide us with better treatment options for patients with DR.

## CONCLUSION

With the introduction of potent pharmacotherapy we have witnessed dramatic improvements in the treatment of DR over the past decade. As we better understand the capabilities of available drugs and integrate them with treatments such as laser and surgery, and add new pharmacologic drugs to our treatment paradigms when they receive FDA approval, the future treatment for DR appears increasingly promising.

## REFERENCES

- 1 Sepúlveda J, Murray C. The state of global health in 2014. *Science* 2014; **345**: 1275-1278 [PMID: 25214611 DOI: 10.1126/science.1257099]
- 2 Fong DS, Aiello LP, Ferris FL, Klein R. Diabetic retinopathy. *Diabetes Care* 2004; **27**: 2540-2553 [PMID: 15451934]
- 3 Nguyen QD, Brown DM, Marcus DM, Boyer DS, Patel S, Feiner L, Gibson A, Sy J, Rundle AC, Hopkins JJ, Rubio RG, Ehrlich JS; RISE and RIDE Research Group. Ranibizumab for diabetic macular edema: results from 2 phase III randomized trials: RISE and RIDE. *Ophthalmology* 2012; **119**: 789-801 [PMID: 22330964 DOI: 10.1016/j.ophtha.2011.12.039]
- 4 Korobelnik JF, Do DV, Schmidt-Erfurth U, Boyer DS, Holz FG, Heier JS, Midena E, Kaiser PK, Terasaki H, Marcus DM, Nguyen QD, Jaffe GJ, Slakter JS, Simader C, Soo Y, Schmelter T, Yancopoulos GD, Stahl N, Vitti R, Berliner AJ, Zeitz O, Metzger C, Brown DM. Intravitreal aflibercept for diabetic macular edema. *Ophthalmology* 2014; **121**: 2247-2254 [PMID: 25012934 DOI: 10.1016/j.ophtha.2014.05.006]



- 5 **Writing Committee for the Diabetic Retinopathy Clinical Research Network**, Gross JG, Glassman AR, Jampol LM, Inusah S, Aiello LP, Antoszyk AN, Baker CW, Berger BB, Bressler NM, Browning D, Elman MJ, Ferris FL, Friedman SM, Marcus DM, Melia M, Stockdale CR, Sun JK, Beck RW. Panretinal Photocoagulation vs Intravitreal Ranibizumab for Proliferative Diabetic Retinopathy: A Randomized Clinical Trial. *JAMA* 2015; **314**: 2137-2146 [PMID: 26565927 DOI: 10.1001/jama.2015.15217]
- 6 Photocoagulation for diabetic macular edema. Early Treatment Diabetic Retinopathy Study report number 1. Early Treatment Diabetic Retinopathy Study research group. *Arch Ophthalmol* 1985; **103**: 1796-1806 [PMID: 2866759]
- 7 Screening for retinopathy in the pediatric patient with type 1 diabetes mellitus. American Academy of Pediatrics. Sections on Endocrinology and Ophthalmology. *Pediatrics* 1998; **101**: 313-314 [PMID: 9457160]
- 8 **American Academy of Ophthalmology Retina Panel**. Preferred Practice Pattern Guidelines. Diabetic Retinopathy. San Francisco, CA: American Academy of Ophthalmology, 2008
- 9 **Fong DS**, Aiello L, Gardner TW, King GL, Blankenship G, Cavallerano JD, Ferris FL, Klein R; American Diabetes Association. Diabetic retinopathy. *Diabetes Care* 2003; **26** Suppl 1: S99-S102 [PMID: 12502630]
- 10 **Canadian Ophthalmological Society Diabetic Retinopathy Clinical Practice Guideline Expert Committee**, Hooper P, Boucher MC, Cruess A, Dawson KG, Delpero W, Greve M, Kozusek V, Lam WC, Maberley DA. Canadian Ophthalmological Society Evidence-based Clinical Practice Guidelines for the Management of Diabetic Retinopathy - executive summary. *Can J Ophthalmol* 2012; **47**: 91-96 [PMID: 22560411 DOI: 10.1016/j.cjco.2012.01.022]
- 11 **Mukamel DB**, Bresnick GH, Wang Q, Dickey CF. Barriers to compliance with screening guidelines for diabetic retinopathy. *Ophthalmic Epidemiol* 1999; **6**: 61-72 [PMID: 10384685]
- 12 **Thompson AC**, Thompson MO, Young DL, Lin RC, Sanislo SR, Moshfeghi DM, Singh K. Barriers to Follow-Up and Strategies to Improve Adherence to Appointments for Care of Chronic Eye Diseases. *Invest Ophthalmol Vis Sci* 2015; **56**: 4324-4331 [PMID: 26176869 DOI: 10.1167/iov.15-16444]
- 13 **Hipwell AE**, Sturt J, Lindenmeyer A, Stratton I, Gadsby R, O'Hare P, Scanlon PH. Attitudes, access and anguish: a qualitative interview study of staff and patients' experiences of diabetic retinopathy screening. *BMJ Open* 2014; **4**: e005498 [PMID: 25510885 DOI: 10.1136/bmjopen-2014-005498]
- 14 **Palmer JJ**, Chinanayi F, Gilbert A, Pillay D, Fox S, Jaggernath J, Naidoo K, Graham R, Patel D, Blanchet K. Trends and implications for achieving VISION 2020 human resources for eye health targets in 16 countries of sub-Saharan Africa by the year 2020. *Hum Resour Health* 2014; **12**: 45 [PMID: 25128287 DOI: 10.1186/1478-4491-12-45]
- 15 **Rajalakshmi R**, Arulmalar S, Usha M, Prathiba V, Kareemuddin KS, Anjana RM, Mohan V. Validation of Smartphone Based Retinal Photography for Diabetic Retinopathy Screening. *PLoS One* 2015; **10**: e0138285 [PMID: 26401839 DOI: 10.1371/journal.pone.0138285]
- 16 **Walton OB**, Garoon RB, Weng CY, Gross J, Young AK, Camero KA, Jin H, Carvounis PE, Coffee RE, Chu YI. Evaluation of Automated Teleretinal Screening Program for Diabetic Retinopathy. *JAMA Ophthalmol* 2016; **134**: 204-209 [PMID: 26720694 DOI: 10.1001/jamaophthalmol.2015.5083]
- 17 **Jiayi Wu**, Jingmin Xin, Lai Hong, You J, Nanning Zheng. New hierarchical approach for microaneurysms detection with matched filter and machine learning. *Conf Proc IEEE Eng Med Biol Soc* 2015; **2015**: 4322-4325 [PMID: 26737251 DOI: 10.1109/EMBC.2015.7319351]
- 18 **Schmidt-Erfurth U**, Lang GE, Holz FG, Schlingemann RO, Lanzetta P, Massin P, Gerstner O, Bouazza AS, Shen H, Osborne A, Mitchell P; RESTORE Extension Study Group. Three-year outcomes of individualized ranibizumab treatment in patients with diabetic macular edema: the RESTORE extension study. *Ophthalmology* 2014; **121**: 1045-1053 [PMID: 24491642 DOI: 10.1016/j.ophtha.2013.11.041]
- 19 **Boyer DS**, Nguyen QD, Brown DM, Basu K, Ehrlich JS; RIDE and RISE Research Group. Outcomes with As-Needed Ranibizumab after Initial Monthly Therapy: Long-Term Outcomes of the Phase III RIDE and RISE Trials. *Ophthalmology* 2015; **122**: 2504-13.e1 [PMID: 26452713 DOI: 10.1016/j.ophtha.2015.08.006]
- 20 **Elman MJ**, Bressler NM, Qin H, Beck RW, Ferris FL, Friedman SM, Glassman AR, Scott IU, Stockdale CR, Sun JK. Expanded 2-year follow-up of ranibizumab plus prompt or deferred laser or triamcinolone plus prompt laser for diabetic macular edema. *Ophthalmology* 2011; **118**: 609-614 [PMID: 21459214 DOI: 10.1016/j.ophtha.2010.12.033]
- 21 **Wells JA**, Glassman AR, Ayala AR, Jampol LM, Aiello LP, Antoszyk AN, Arnold-Bush B, Baker CW, Bressler NM, Browning DJ, Elman MJ, Ferris FL, Friedman SM, Melia M, Pieramici DJ, Sun JK, Beck RW. Aflibercept, bevacizumab, or ranibizumab for diabetic macular edema. *N Engl J Med* 2015; **372**: 1193-1203 [PMID: 25692915 DOI: 10.1016/j.ophtha.2016.02.022]
- 22 **CATT Research Group**, Martin DF, Maguire MG, Ying GS, Grunwald JE, Fine SL, Jaffe GJ. Ranibizumab and bevacizumab for neovascular age-related macular degeneration. *N Engl J Med* 2011; **364**: 1897-1908 [PMID: 21526923 DOI: 10.1056/NEJMoa1102673]
- 23 **Stone TW**, editor. American Society of Retina Specialists 2015 Preferences and Trends Membership Survey. Chicago, IL: American Society of Retina Specialists, 2015
- 24 **Prünte C**, Fajnkuchen F, Mahmood S, Ricci F, Hatz K, Studnička J, Bezlyak V, Parikh S, Stubbings WJ, Wenzel A, Figueira J; RETAIN Study Group. Ranibizumab 0.5 mg treat-and-extend regimen for diabetic macular oedema: the RETAIN study. *Br J Ophthalmol* 2016; **100**: 787-795 [PMID: 26453639 DOI: 10.1136/bjophthalmol-2015-307249]
- 25 **Heier JS**, Bressler NM, Avery RL, Bakri SJ, Boyer DS, Brown DM, Dugel PU, Freund KB, Glassman AR, Kim JE, Martin DF, Pollack JS, Regillo CD, Rosenfeld PJ, Schachat AP, Wells JA. Comparison of Aflibercept, Bevacizumab, and Ranibizumab for Treatment of Diabetic Macular Edema: Extrapolation of Data to Clinical Practice. *JAMA Ophthalmol* 2016; **134**: 95-99 [PMID: 26512939 DOI: 10.1001/jamaophthalmol.2015.4110]
- 26 **Boyer DS**, Yoon YH, Belfort R, Bandello F, Maturi RK, Augustin AJ, Li XY, Cui H, Hashad Y, Whitcup SM; Ozurdex MEAD Study Group. Three-year, randomized, sham-controlled trial of dexamethasone intravitreal implant in patients with diabetic macular edema. *Ophthalmology* 2014; **121**: 1904-1914 [PMID: 24907062 DOI: 10.1016/j.ophtha.2014.04.024]
- 27 **Campochiaro PA**, Brown DM, Pearson A, Chen S, Boyer D, Ruiz-Moreno J, Garretson B, Gupta A, Hariprasad SM, Bailey C, Reichel E, Soubrane G, Kapik B, Billman K, Kane FE, Green K. Sustained delivery fluocinolone acetonide vitreous inserts provide benefit for at least 3 years in patients with diabetic macular edema. *Ophthalmology* 2012; **119**: 2125-2132 [PMID: 22727177 DOI: 10.1016/j.ophtha.2012.04.030]
- 28 **Cunha-Vaz J**, Ashton P, Iezzi R, Campochiaro P, Dugel PU, Holz FG, Weber M, Danis RP, Kuppermann BD, Bailey C, Billman K, Kapik B, Kane F, Green K. Sustained delivery fluocinolone acetonide vitreous implants: long-term benefit in patients with chronic diabetic macular edema. *Ophthalmology* 2014; **121**: 1892-1903 [PMID: 24935282 DOI: 10.1016/j.ophtha.2014.04.019]
- 29 **Diabetic Retinopathy Clinical Research Network**, Elman MJ, Qin H, Aiello LP, Beck RW, Bressler NM, Ferris FL, Glassman AR, Maturi RK, Melia M. Intravitreal ranibizumab for diabetic macular edema with prompt versus deferred laser treatment: three-year randomized trial results. *Ophthalmology* 2012; **119**: 2312-2318 [PMID: 22999634 DOI: 10.1016/j.ophtha.2012.08.022]
- 30 **Peden MC**, Suñer JJ, Hammer ME, Grizzard WS. Long-term outcomes in eyes receiving fixed-interval dosing of anti-vascular endothelial growth factor agents for wet age-related macular degeneration. *Ophthalmology* 2015; **122**: 803-808 [PMID: 25596618 DOI: 10.1016/j.ophtha.2014.11.018]
- 31 **Campochiaro PA**, Channa R, Berger BB, Heier JS, Brown DM, Fiedler U, Hepp J, Stumpp MT. Treatment of diabetic macular

- edema with a designed ankyrin repeat protein that binds vascular endothelial growth factor: a phase I/II study. *Am J Ophthalmol* 2013; **155**: 697-704, 704.e1-2 [PMID: 23218689 DOI: 10.1016/j.ajo.2012.09.032]
- 32 **Allergan**. Safety and Efficacy of Abicipar Pegol in Patients With Neovascular Age-related Macular Degeneration. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). [accessed 2016 Jan 24]. Available from: URL: <https://clinicaltrials.gov/ct2/show/NCT02462486> NLM Identifier: NCT02462486
  - 33 **Helzner J**. Promising Data on Sustained-release Lucentis. [accessed 2016 Jan 24]. Available from: URL: <http://www.retinalphysician.com/articleviewer.aspx?articleID=111136>
  - 34 **Neurotech Pharmaceuticals**. Study of the Intravitreal Implantation of NT-503-3 Encapsulated Cell Technology (ECT) for the Treatment of Recurrent Choroidal Neovascularization (CNV) Secondary to Age-related Macular Degeneration (AMD). In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). [accessed 2016 Jan 24]. Available from: URL: <https://clinicaltrials.gov/ct2/show/NCT02228304> NLM Identifier: NCT02228304
  - 35 **Kauper K**, McGovern C, Sherman S, Heatherton P, Rapoza R, Stabila P, Dean B, Lee A, Borges S, Bouchard B, Tao W. Two-year intraocular delivery of ciliary neurotrophic factor by encapsulated cell technology implants in patients with chronic retinal degenerative diseases. *Invest Ophthalmol Vis Sci* 2012; **53**: 7484-7491 [PMID: 23049090 DOI: 10.1167/iovs.12-9970]
  - 36 **Diabetic Retinopathy Clinical Research Network**. Treatment for CI-DME in Eyes With Very Good VA Study (Protocol V). In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). [accessed 2016 Jan 24]. Available from: URL: <https://clinicaltrials.gov/ct2/show/NCT01909791> NLM Identifier: NCT01909791
  - 37 Photocoagulation treatment of proliferative diabetic retinopathy. Clinical application of Diabetic Retinopathy Study (DRS) findings, DRS Report Number 8. The Diabetic Retinopathy Study Research Group. *Ophthalmology* 1981; **88**: 583-600 [PMID: 7196564]
  - 38 **Avery RL**. Regression of retinal and iris neovascularization after intravitreal bevacizumab (Avastin) treatment. *Retina* 2006; **26**: 352-354 [PMID: 16508438]
  - 39 **Spaide RF**, Fisher YL. Intravitreal bevacizumab (Avastin) treatment of proliferative diabetic retinopathy complicated by vitreous hemorrhage. *Retina* 2006; **26**: 275-278 [PMID: 16508426]
  - 40 **Jorge R**, Costa RA, Calucci D, Cintra LP, Scott IU. Intravitreal bevacizumab (Avastin) for persistent new vessels in diabetic retinopathy (IBEPE study). *Retina* 2006; **26**: 1006-1013 [PMID: 17151487]
  - 41 **Zhang ZH**, Liu HY, Hernandez-Da Mota SE, Romano MR, Falavarjani KG, Ahmadi H, Xu X, Liu K. Vitrectomy with or without preoperative intravitreal bevacizumab for proliferative diabetic retinopathy: a meta-analysis of randomized controlled trials. *Am J Ophthalmol* 2013; **156**: 106-115.e2 [PMID: 23791371 DOI: 10.1016/j.ajo.2013.02.008]
  - 42 **Mitchell P**, Bandello F, Schmidt-Erfurth U, Lang GE, Massin P, Schlingemann RO, Sutter F, Simader C, Burian G, Gerstner O, Weichselberger A. The RESTORE study: ranibizumab monotherapy or combined with laser versus laser monotherapy for diabetic macular edema. *Ophthalmology* 2011; **118**: 615-625 [PMID: 21459215 DOI: 10.1016/j.ophtha.2011.01.031]
  - 43 **Nicolò M**, Musetti D, Traverso CE. Yellow micropulse laser in diabetic macular edema: a short-term pilot study. *Eur J Ophthalmol* 2014; **24**: 885-889 [PMID: 24905254 DOI: 10.5301/ejo.5000495]
  - 44 **Kwon YH**, Lee DK, Kwon OW. The short-term efficacy of subthreshold Micropulse yellow (577-nm) laser photocoagulation for diabetic macular edema. *Korean J Ophthalmol* 2014; **28**: 379-385 [PMID: 25276079 DOI: 10.3341/kjo.2014.28.5.379]
  - 45 **Suñer IJ**, Peden MC, Hammer ME, Grizzard WS, Traynom J, Cousins SW. RaScaL: A Pilot Study to Assess the Efficacy, Durability, and Safety of a Single Intervention with Ranibizumab plus Peripheral Laser for Diabetic Macular Edema Associated with Peripheral Nonperfusion on Ultrawide-Field Fluorescein Angiography. *Ophthalmologica* 2014; Epub ahead of print [PMID: 25427532]
  - 46 **Lim LS**, Ng WY, Mathur R, Wong D, Wong EY, Yeo I, Cheung CM, Lee SY, Wong TY, Papakostas TD, Kim LA. Conversion to aflibercept for diabetic macular edema unresponsive to ranibizumab or bevacizumab. *Clin Ophthalmol* 2015; **9**: 1715-1718 [PMID: 26396494 DOI: 10.2147/OPHT.S81523]
  - 47 **Maturi RK**, Bleau L, Saunders J, Mubasher M, Stewart MW. A 12-month, single-masked, randomized controlled study of eyes with persistent diabetic macular edema after multiple anti-VEGF injections to assess the efficacy of the dexamethasone-delayed delivery system as an adjunct to bevacizumab compared with continued bevacizumab monotherapy. *Retina* 2015; **35**: 1604-1614 [PMID: 25829346 DOI: 10.1097/IAE.0000000000000533]
  - 48 **Lewis H**, Abrams GW, Blumenkranz MS, Campo RV. Vitrectomy for diabetic macular traction and edema associated with posterior hyaloidal traction. *Ophthalmology* 1992; **99**: 753-759 [PMID: 1594222]
  - 49 **Landers MB III**, Kon Graversen VA, Stewart MW. Early vitrectomy for DME: does it have a role? Sometimes vitrectomy can be first-line treatment. Part 1 of 2. *Retinal Physician*, 2013: 46-53
  - 50 **Landers MB III**, Kon Graversen VA, Stewart MW. Early vitrectomy for DME: does it have a role? Sometimes vitrectomy can be first-line treatment. Part 2 of 2. *Retinal Physician*, 2013: 56-60
  - 51 **Simunovic MP**, Hunyor AP, Ho IV. Vitrectomy for diabetic macular edema: a systematic review and meta-analysis. *Can J Ophthalmol* 2014; **49**: 188-195 [PMID: 24767227 DOI: 10.1016/j.jcjo.2013.11.012]
  - 52 **Flaxel CJ**, Edwards AR, Aiello LP, Arrigg PG, Beck RW, Bressler NM, Bressler SB, Ferris FL, Gupta SK, Haller JA, Lazarus HS, Qin H. Factors associated with visual acuity outcomes after vitrectomy for diabetic macular edema: diabetic retinopathy clinical research network. *Retina* 2010; **30**: 1488-1495 [PMID: 20924264 DOI: 10.1097/IAE.0b013e3181e7974f]
  - 53 **Chhablani JK**, Kim JS, Cheng L, Kozak I, Freeman W. External limiting membrane as a predictor of visual improvement in diabetic macular edema after pars plana vitrectomy. *Graefes Arch Clin Exp Ophthalmol* 2012; **250**: 1415-1420 [PMID: 22354371 DOI: 10.1007/s00417-012-1968-x]

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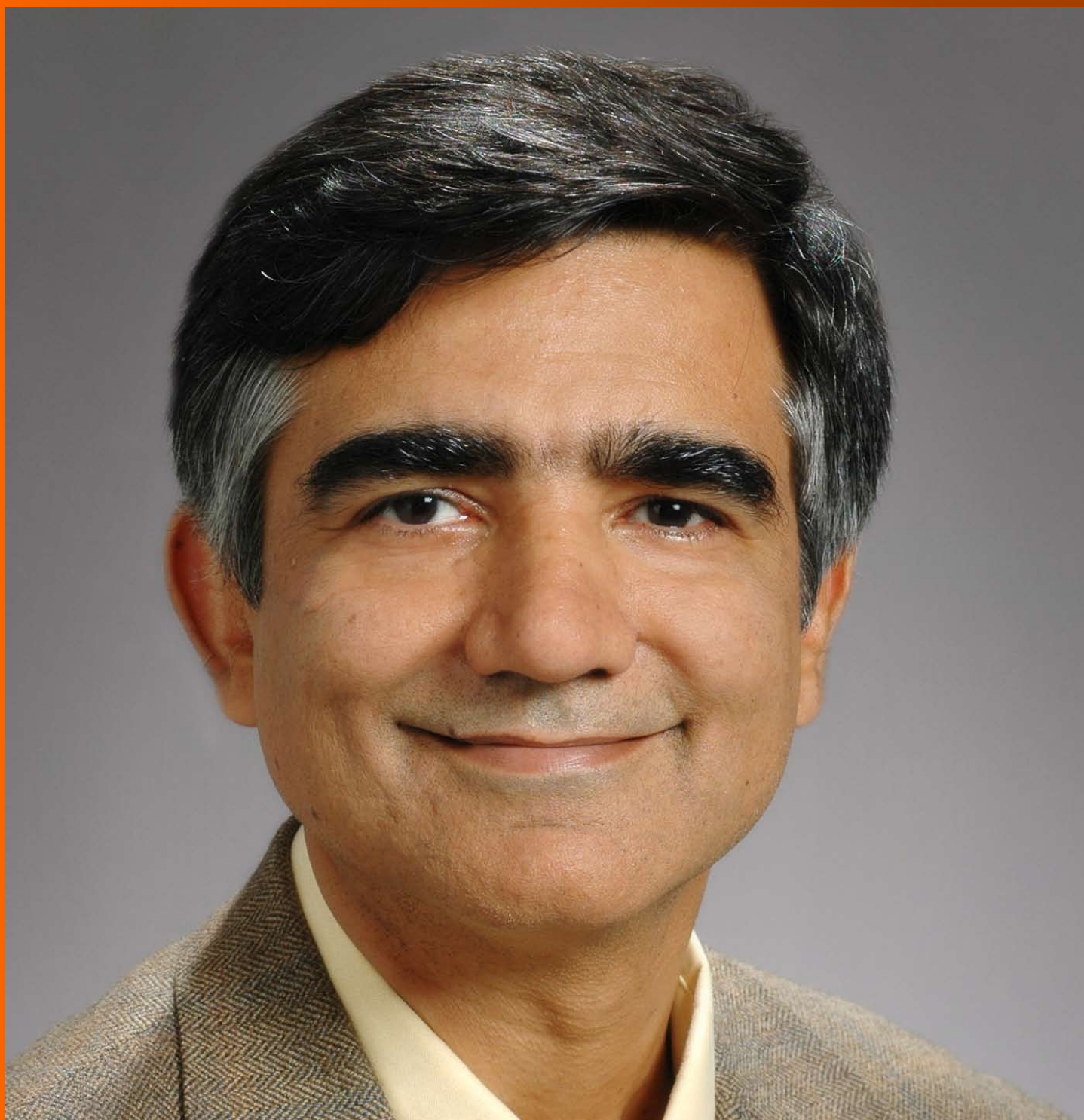
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## Entrapment neuropathies in diabetes mellitus

Eugenia Rota, Nicola Morelli

Eugenia Rota, Nicola Morelli, Department of Neurology, Ospedale G. da Saliceto, 29121 Piacenza, Italy

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**Correspondence to:** Eugenia Rota, MD, Department of Neurology, Ospedale G. da Saliceto, Via Taverna 49, 29121 Piacenza, Italy. [eugenia.rota.md@gmail.com](mailto:eugenia.rota.md@gmail.com)  
Telephone: +39-0523-303310  
Fax: +39-0523-303322

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### Abstract

Neuropathy is a common complication of diabetes mellitus (DM) with a wide clinical spectrum that encompasses generalized to focal and multifocal forms. Entrapment neuropathies (EN), which are focal forms, are so frequent at any stage of the diabetic disease, that they may be considered a neurophysiological hallmark

of peripheral nerve involvement in DM. Indeed, EN may be the earliest neurophysiological abnormalities in DM, particularly in the upper limbs, even in the absence of a generalized polyneuropathy, or it may be superimposed on a generalized diabetic neuropathy. This remarkable frequency of EN in diabetes is underlain by a peculiar pathophysiological background. Due to the metabolic alterations consequent to abnormal glucose metabolism, the peripheral nerves show both functional impairment and structural changes, even in the preclinical stage, making them more prone to entrapment in anatomically constrained channels. This review discusses the most common and relevant EN encountered in diabetic patient in their epidemiological, pathophysiological and diagnostic features.

**Key words:** Diabetes mellitus; Neuropathy; Diabetic neuropathy; Median entrapment neuropathy at the wrist; Ulnar entrapment neuropathy at the elbow; Ulnar entrapment neuropathy at the wrist; Carpal tunnel syndrome; Electrodiagnosis; Tarsal tunnel syndrome

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**Core tip:** Diabetic neuropathy syndromes include both generalized and focal/multifocal forms. Entrapment neuropathies (EN) are remarkably frequent in the focal forms and may be the earliest neurophysiological abnormalities in diabetes, even in the absence of a generalized polyneuropathy. Based on a pathophysiological hypothesis, diabetic patients' peripheral nerves, damaged by the altered glucose metabolism, show both functional impairment and structural changes. This makes them more susceptible to chronic compression in anatomically constrained channels. Therefore, EN may be considered a neurophysiological hallmark of peripheral nerve involvement in diabetes mellitus.

Rota E, Morelli N. Entrapment neuropathies in diabetes mellitus. *World J Diabetes* 2016; 7(17): 342-353 Available from: URL:

## INTRODUCTION

Neuropathy is a major complication of diabetes mellitus (DM) and is as common as protean. Moreover, it not only leads to an impaired quality of life, but also to an increased morbidity and mortality<sup>[1]</sup>. Diabetic neuropathy (DN) is the most common form of neuropathy in Western countries, with a wide prevalence in literature, ranging from 5% to 90%<sup>[2,3]</sup>. Such a large discrepancy is mainly due to the different methods, *i.e.*, sets of electrophysiological and clinical criteria and demographic data adopted<sup>[4]</sup>. The EURODIAB IDDM Complication Study reports a 28% prevalence of DN across Europe<sup>[4]</sup>. Considering that DM affects about 246 million people worldwide, it can be estimated that 20-30 million people have a DN<sup>[5]</sup>.

There are numerous and heterogeneous neuropathic syndromes associated with DM. Indeed, the Toronto Diabetic Neuropathy Expert Group<sup>[1,6]</sup> updated the classification of DNs, providing definitions, minimal criteria for diagnoses and severity estimations. The Toronto Consensus Panels on DN<sup>[1,6]</sup> accepted Thomas<sup>[7]</sup> and Boulton *et al.*<sup>[8]</sup>'s separation of DNs into generalized and focal/multifocal neuropathies (Table 1).

Generalized DNs can be further classified into at least two major subgroups, *i.e.*, typical and atypical<sup>[1,6]</sup>. The most common generalized DN is the typical symmetric sensorimotor polyneuropathy, which is known to be length-dependent<sup>[9]</sup>. The atypical forms of generalized DN are usually intercurrent, painful varieties and can develop acutely at any time during the course of a patient's disease<sup>[10]</sup>.

### **Focal and multifocal neuropathies in DM: Entrapment syndromes vs mononeurites/multiple mononeuropathies**

The focal and multifocal neuropathies associated with DM can be broadly subdivided into two categories<sup>[6]</sup>, which are characterized by different clinical features and underpinned by various pathophysiological backgrounds. Entrapment of the nerve, favoured by chronic compression, is the pivotal causative factor in the first group: This includes median neuropathy at the wrist (MNW), ulnar neuropathy at the elbow (UNE) and peroneal neuropathy at the knee<sup>[6]</sup>. The second group encompasses cranial mononeuropathies or mononeurites, multiple mononeuropathies and thoracic, cervical and, most frequently, lumbosacral radiculoplexus neuropathies. The latter is also known as diabetic amyotrophy or Bruns Garland syndrome, where the pathophysiology involves inflammatory factors, microvasculitis and ischemia<sup>[11-13]</sup>. The 3<sup>rd</sup> and 6<sup>th</sup> are the most commonly affected cranial nerves, whilst the 7<sup>th</sup> is more rarely involved. Some pathological studies have demonstrated a centro-fascicular lesion in the

**Table 1 Diabetic neuropathies classification (according to Dyck *et al.*<sup>[6]</sup>, on behalf of the Toronto Expert Panel on Diabetic Neuropathy, 2011)**

Diabetic neuropathies	
Generalized	Typical (symmetrical, length-dependent, sensorimotor neuropathy) Atypical (painful varieties)
Focal/multifocal	Multiple mononeuropathy Entrapment neuropathy Lumbosacral/thoracic/cervical radiculoplexus neuropathy

intracavernous portion of the 3<sup>rd</sup> cranial nerve, sparing the peripheral fibres (and, consequently, the pupillary reaction), attributable to ischemic damage<sup>[5,14]</sup>.

The two groups, *i.e.*, entrapment neuropathies (EN) and mononeuropathies/multiple mononeuropathies, have different clinical characteristics<sup>[15]</sup>. Indeed, mononeurites or multiple mononeuropathies usually have an acute onset, where pain is a common feature, and a self-limiting clinical course within a 6-mo period, even if forms like diabetic amyotrophy may be highly disabling. On the other hand, EN have a gradual onset, a slow progression and persist without intervention<sup>[15]</sup>.

### **EN: General epidemiological remarks**

EN are remarkably common in DM<sup>[15,16]</sup> at any stage and may be asymptomatic. Therefore, patients with signs and symptoms suggestive of an entrapment should be thoroughly investigated, as surgery may be indicated<sup>[15]</sup>.

A study by Stamboulis *et al.*<sup>[17]</sup> aimed at establishing whether symptomatic mononeuropathies are more frequent in diabetic patients without symptoms of polyneuropathy than in the general population. A large cohort of 642 consecutive outpatients with various acute symptomatic mononeuropathies (radial, ulnar or peroneal neuropathy, Bell's palsy or median neuropathy at the carpal tunnel) were screened for the presence of DM. The results showed that in 522/642 patients with symptomatic carpal tunnel syndrome (CTS) and in 38/522 with Bell's palsy, DM frequency (7.7% and 10.5%, respectively) did not differ significantly from that expected in the general population<sup>[17]</sup>. Conversely, the respective DM rates (27.8%, 12.2% and 30.4%) were significantly higher than in the general population in 18 patients with radial neuropathy at (or distally to) the spiral groove<sup>[17]</sup>. The same finding was observed in 41 patients with ulnar neuropathy and in 23 patients with peroneal neuropathy at the fibular head<sup>[17]</sup>. This suggests that diabetic patients are more prone to focal limb neuropathies caused by acute external compression. However, this study focused on acute symptomatic mononeuropathies, whilst the majority of EN in DM are chronic and often asymptomatic.

Some cross-sectional and population studies reported a high prevalence of both symptomatic and asymptomatic MNW and ulnar nerve entrapments, with an increased lifetime risk for CTS, compared to the general

population. Herein, we should only like to emphasize that the presence of MNW was detected in 28% of DM patients at diagnosis<sup>[18]</sup> and that this proportion rose to 62.5% in patients with an average disease duration of 14.5 years<sup>[19]</sup>. This finding seems to confirm an association between EN, previously reported as not being age-dependent<sup>[20]</sup>, and longer disease duration. Furthermore, subclinical UNE was electro-diagnosed in a remarkably high percentage, *i.e.*, 34%, of DM patients<sup>[19]</sup>, suggesting that the ulnar nerve is very susceptible to focal entrapment in DM, as is the median nerve. These findings and others (see CTS and ulnar entrapments), suggest that EN in DM, mostly at the upper limbs, are not late complications, but rather early neurophysiological abnormalities, where the frequency increases with the disease duration and/or in the presence of generalized DN.

## THE PATHOPHYSIOLOGY OF EN: OLD AND NEW EVIDENCE

Epidemiological findings suggest that peripheral nerves are strikingly susceptible to focal entrapment in the presence of DM. Such a liability to chronic compression in DM may be attributable to metabolic factors and endoneurial ischemia, which damage the nerves already in the long preclinical stage<sup>[21]</sup>, as was first proven in animal models<sup>[22]</sup>. Therefore, a focal EN may be the first and, at times, only manifestation of a peripheral nerve involvement not only in DM, but also in prediabetes.

Growing evidence has shown that, on the one hand, impaired glucose tolerance may cause peripheral neuropathy itself and, on the other, abnormal glucose metabolism underlies a relevant proportion of apparently "idiopathic" sensory neuropathies<sup>[23-25]</sup>. The effects of sustained impaired glucose tolerance and progressive insulinopenia, also in the absence of hyperglycemia, were studied in an animal model (Goto-Kakizaki rat), which showed a functional and structural neuropathy associated with impaired NGF support and neuropeptide synthesis<sup>[26]</sup>. Indeed, insulin deficiency has been proven to be a pivotal pathogenetic factor in DN, owing to its unique trophic properties that act on sensory neuron and axon receptors<sup>[27]</sup>. Not only has it been shown that abnormal direct neuronal insulin signaling contributes to neuro-degeneration, but studies are ongoing on other important molecular factors that influence neuronal and axon growth, such as PTEN (phosphatase and tensin homolog deleted on chromosome 10)<sup>[28]</sup>.

On the other hand, observational studies have reported an increased prevalence of impaired glucose tolerance (up to 34%) in subjects with painful sensory neuropathy<sup>[23,24]</sup>. In another study, where patients with peripheral neuropathy of unknown origin were administered Oral Glucose Tolerance Test, 56% had abnormal results. Moreover, patients with impaired glucose tolerance had predominantly small fibre neuropathy, compared to those with overt DM, who

showed more prominent involvement of large fibres<sup>[25]</sup>. Hence, some kind of a "continuum" of peripheral nerve damage, associated with glucose dysmetabolism, may be hypothesized. A subtle impairment of nerve function, which begins in the preclinical stage of DM and progresses into the more advanced stages of the disease, is involved in this dysmetabolism, where sustained hyperglycemia alters biochemical pathways in the neurons, making the nerves more susceptible to entrapment.

There is clear evidence of the pathogenetic role the activated polyol pathway plays in diabetic nerves. Both the neurons and Schwann cells of patients with chronic hyperglycemia undergo a shift from the physiologic conversion of glucose into glucose-6-phosphate by hexokinase into an alternative pathway, where excess glucose is transformed into sorbitol by the aldose-reductase. Sorbitol, due to its low plasma membrane permeability, may act as an osmotic driver and, consequently, promote axonal and nerve trunk swelling in DM<sup>[29]</sup>. Moreover, the activated polyol pathway may induce a decrease in Na/K ATPase activity, leading to intra-axonal Na accumulation and a reduced Na gradient across the plasmatic membrane<sup>[30]</sup>.

Prolonged hyperglycemia may also enhance oxidative stress as radical scavengers are recharged too slowly to counterbalance the higher activity of the electron transport chain induced by the glucose overload<sup>[29]</sup>. The nerve axons, which are rich in mitochondria, are particularly vulnerable to oxidative damage in DM. Such a "double cellular crisis" of energy failure and oxidative damage has also been proven in Schwann cells<sup>[31]</sup>.

Furthermore, neurodegeneration may also be promoted by advanced glycation end products, which accumulate due to the non-enzymatic glycosylation of proteins and may even damage the function of pericytes and impair the nerve vascular supply<sup>[29]</sup>. A study on an animal model also led to the hypothesis that endoneurium and perineurium metabolic and phenotypic abnormalities may be underlying causal factors in the high sensitivity of diabetic nerves to entrapment<sup>[32]</sup>.

### The "double crush" hypothesis revisited

All these metabolic alterations lead to both functional impairment and structural changes, mainly swelling, in the nerves, making them more prone to entrapment in anatomically constrained channels<sup>[33]</sup>. In other words, there is a sort of "two hit" model. The glucose derangement hits the peripheral nerve first, which then becomes more susceptible to a second "hit", by the local factors related to entrapment, such as increased pressure, strain and/or elongation in the anatomically narrow sites. This may well be in agreement with Upton's "double crush hypothesis". In 1973, Drs. Upton and McComas<sup>[34]</sup> hypothesized in the journal *Lancet* that, if non-symptomatic impairment of axoplasmic flow occurs at more than one site along a nerve, it might well sum-up to cause a symptomatic neuropathy<sup>[33,35]</sup>. This

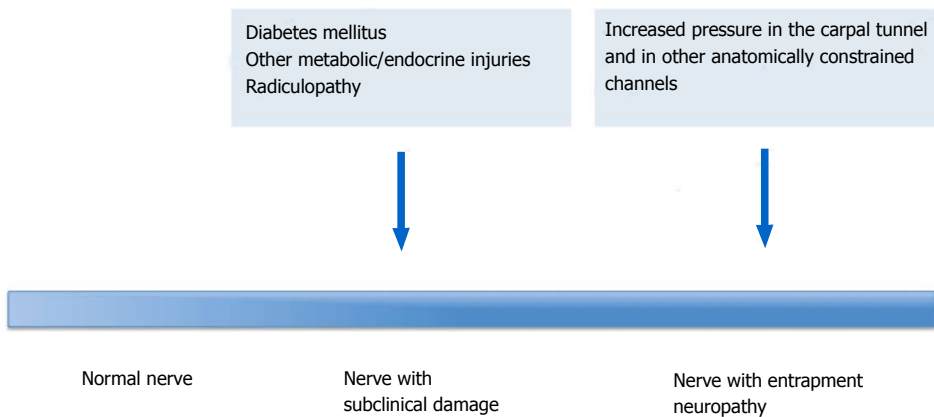


Figure 1 The “Double Crush” hypothesis revisited.

hypothesis relied on the clinical observation that most patients observed by the authors had an MNW, which was often bilateral, or an ulnar neuropathy, associated with cervical radiculopathy. Drs. Upton and McComas<sup>[34]</sup> supposed that axoplasmic flow could also be impaired by the metabolic damage, based on the frequent association between DM and CTS. Hence, in a revisited and extended “double crush hypothesis” (Figure 1), not only proximal nerve impingement, but also metabolic dysfunction and nerve swelling subsequent to DN, may be involved in the “first crush”. This predisposes nerves to chronic compression, *i.e.*, the “second crush”, in anatomically constrained sites, like carpal and/or tarsal tunnels<sup>[15]</sup>.

This mechanism seems to be consistent with the frequent association of both generalized and focal DN, where an EN is superimposed on a generalized form of DN. Indeed, the metabolic derangement and the microvessel alterations, subsequent to chronic hyperglycemia, lead to exhaustion of the ATP supply and an earlier fibre dissolution in the distal nerve compartment<sup>[36]</sup>. This may be in line with the well-known dying-back pathophysiological mechanisms that underlie generalized typical, symmetrical, length-dependent diabetic neuropathy. However, the same subclinical damage to distal nerve segments and the early demyelination of small sensory fibres may be taken into account when interpreting a slow conduction velocity and/or conduction blocks across the carpal tunnel, in the distal median nerve in MNW, which is often the earliest EN.

#### **Diabetic neuropathy: Axonal or demyelinating?**

The question thus arises whether the nature of DN is axonal or demyelinating. The results of a study aimed at answering this question<sup>[37]</sup> suggested that both mechanisms are involved. Demyelination seems to appear earlier in diabetic patients with or without symptoms of polyneuropathy, whilst axonal loss seems prevalent in more advanced DN, where it may be responsible for most of the symptoms. Moreover, the abnormal conduction velocity in the distal segment of the

sural nerve, observed by Cappellari *et al.*<sup>[38]</sup> in impaired glucose tolerance subjects without clinical neuropathy, also suggests that the myelin dysfunction of the distal sensory fibres represents the earliest detectable nerve response to hyperglycemia. Furthermore, a peculiar pattern of “abnormal median or ulnar/normal sural”, *i.e.*, reduced sensory action potential of median or ulnar nerve in the presence of normal sensory action potential of sural nerve, was detected in 82% and 80% respectively of newly diagnosed diabetic patients<sup>[18]</sup>. Such a peculiar pattern was found in a high proportion of patients with acute inflammatory polyneuropathy and was thus considered suggestive of an early distal nerve involvement<sup>[39]</sup>. Hence, if the small myelinated nerve fibres may be assumed to be the most susceptible to entrapment, this may explain the finding of median neuropathy in a very distal segment of the nerve across the carpal tunnel, as the earliest neurophysiological alteration in patients with abnormal glucose metabolism (impaired glucose tolerance and DM). This is observed even in the absence of an overt DN. On the other hand, demyelination has been described as the first human nerve response to chronic compression in the pioneering studies carried out by Dellon *et al.*<sup>[22]</sup> and Mackinnon *et al.*<sup>[40]</sup>, who detected markedly thinner myelin after compressive injuries. A Schwann cell proliferation, accompanied by increased apoptosis, has also been observed in animal models some weeks after compression<sup>[41]</sup>. These and other experimental findings reviewed by Tapadia *et al.*<sup>[41]</sup>, suggest that myelinated neurons may be particularly susceptible to mechanical stress, a pivotal factor in EN. Therefore, in the presence of a DN the peripheral nerves, that are already suffering from endoneurial ischemia and altered axonal excitability, are made more vulnerable to pressure.

This may lead, on the one hand, to an induction of demyelination and, on the other, cause local vascular impairment and superimposed axonal damage in anatomical tunnels<sup>[42]</sup>. This seems to imply that, in EN, once an entrapment has occurred, the chronic compression may enhance the pre-existing nerve metabolic damage within a sort of vicious circle, leading to



worsening, unless surgery is performed. Furthermore, regeneration is impaired in DM patients. Indeed, the microangiopathic changes in small vessels, the metabolic derangement of neurons and Schwann cells, defects in the inflammatory cells within the injury milieu and lack of trophic factors, may contribute to the failure of regenerative programmes<sup>[43]</sup>.

### **EN: Often asymptomatic**

Another peculiar finding of EN in DM deserves mention. It is well known that DN are often asymptomatic. Indeed EN, mainly MNE and UNE, may frequently occur as subclinical neurophysiological alterations, in the absence of clinical symptoms, as demonstrated by some studies<sup>[18,19,44,45]</sup>. MNW was asymptomatic in 36% of the patients in a cohort of some newly diagnosed DM patients<sup>[18]</sup>, similar to that obtained by Celiker *et al.*<sup>[44]</sup>. This suggests that the presence of lesions in the proximal nerve segment and/or an alteration of the threshold of the sensory nerve fibres may render patients with DN less prone to develop a clinically evident CTS than normal controls<sup>[18,20,44,45]</sup>.

## **EN DIAGNOSIS: GENERAL METHODOLOGICAL ASPECTS**

Electro-diagnostic studies are the mainstay in the diagnostic work-up of EN. Sensory and motor conduction studies provide an array of documentation on neuropathy. They distinguish the generalized forms from focal forms and show focal neurophysiologic abnormalities in anatomically constrained channels along the suspected nerve. Moreover, electrodiagnostic studies allow for the demonstration of the axonal or demyelinating features of the neuropathy, the staging of its severity and, last but not least, the exclusion of other concomitant diseases. One remarkable characteristic of electromyography is that it is able to detect a superimposed radiculopathy (such as a cervical C7-C8 radiculopathy concomitant with CTS) in the aforementioned "double crush" syndrome<sup>[34]</sup>.

There is growing evidence in favour of the use of imaging techniques as ancillary or complementary methods in the diagnostic process of neuropathies, above all for EN. Ultrasonography has been proven to offer several advantages in assessing peripheral nerves, including its cost-effectiveness, time-efficient evaluation of long nerve segments, ability to perform dynamic maneuvers, lack of contraindications, portability and non-invasiveness<sup>[46]</sup>. The last decade has witnessed an extensive use of neuromuscular ultrasonography, particularly in the assessment of EN, where the most common and reproducible sonographic finding is nerve enlargement, just proximal to the site of entrapment<sup>[47]</sup>. This enlargement is typically fusiform, rather than discretely focal, and is usually measured by the nerve cross-sectional area. Although the cause of nerve enlargement has not yet been completely clarified, it

has been hypothesized to be the result of axoplasmic damming, as observed in entrapment and chronic nerve compression models<sup>[47]</sup>. Moreover, inflammatory and/or vascular components may contribute to nerve enlargement. Along with nerve enlargement, just proximal to the site of entrapment, other less common findings have been reported and include hypoechoic nerve echo-texture, nerve flattening and pinching at the entrapment site, enlargement of single or multiple fascicles and/or increased vascularity within the nerve<sup>[47]</sup>. A recent study<sup>[48]</sup> was carried out to identify ultrasound findings in type II DM patients complaining of neuropathic symptoms and signs. Nerve ultrasound revealed an increased cross-sectional area in the peripheral nerves both at compression sites, even in the absence of clinical symptoms, and at non-compression sites. The authors hypothesize that cross-sectional area enlargement at compression sites indicates subclinical nerve damage and probably susceptibility to entrapment. Whilst cross-sectional area increase at non-compression sites suggests early morphological abnormalities, even when nerve conduction studies are unremarkable<sup>[48]</sup>. However, further studies should be carried out to confirm these results and to identify any correlations between ultrasonographic and electrodiagnostic findings.

The current role of magnetic resonance imaging (MRI) neurography in diabetic neuropathy is mainly that of excluding the presence of a lesion as the cause of nerve entrapment in cases of focal or regionally distributed multifocal neuropathy, mostly when clinical and electrodiagnostic findings are inconclusive. Furthermore, MRI neurography can diagnose those extra-neural affections that mimic neuropathic symptoms, such as Charcot arthropathy, osteomyelitis, plantar fasciitis, *etc.*<sup>[49]</sup>.

## **MEDIAN ENTRAPMENT NEUROPATHY AT THE WRIST AND CARPAL TUNNEL SYNDROME**

Median nerve entrapment neuropathy at the wrist (MNW) is the prototype of EN and is caused by the compression and traction of the median nerve within the carpal tunnel, an osteofibrous outlet located between the transverse carpal ligament and the carpal bones. It may be asymptomatic or accompanied by sensory complaints (pain, numbness, paraesthesias) or motor symptoms (weakness, clumsiness) in the section of the hand supplied by the median nerve. CTS is the commonest median neuropathy, with a 10% lifetime risk in the general population<sup>[50]</sup>. Prevalence rates vary widely across studies, depending on various factors, such as the geographic area, age, anthropometric data, exposure to risk factors for CTS and the diagnostic criteria used. Recently, a CTS prevalence of 2.3% to 4.3% has been reported in two large cohorts of French workers<sup>[51]</sup>. Some studies<sup>[18-20,42,52]</sup> report the prevalence

of both MNW and CTS to be several-fold higher in DM patients than in the general population, above all in DM patients with polyneuropathy and/or long disease duration. CTS has been detected in 14% of diabetic subjects without polyneuropathy and in 30% of subjects with polyneuropathy<sup>[42]</sup>. Moreover, an MNW was found in 28% of newly diagnosed DM patients, compared to 62.5% of patients with an average disease duration of 14.5 years<sup>[18,19]</sup>. Similar results were reported in another study carried out on 146 DM patients, where CTS was diagnosed in 39% of the sample, 28% of males and 46% of females<sup>[53]</sup>. The risk of hand syndromes, including CTS, stenosing flexor tenosynovitis and Dupuytren disease, was evaluated in a population-based cohort study (606152 diabetic patients and 609970 matched for age and gender)<sup>[54]</sup>, where the hazard ratio for CTS was: 1.31 (95%CI: 1.28-1.34) in DM patients. In the longitudinal Fremantle Diabetes Study, aimed at determining the incidence and predictors of carpal tunnel decompression in 1284 DM patients, the incidence of CTS was 5.5 cases per 1000 patient-year, at least 4.2-fold that of the general population<sup>[52]</sup>. In a previous review, aimed at evidencing any increase in the prevalence of specific conditions in CTS patients, a two-fold increased risk (OR = 2.2; 95%CI: 1.5-3.1) for DM was detected<sup>[55]</sup>. Therefore, DM is an independent risk factor for CTS<sup>[55]</sup>. A surprisingly high lifetime risk of CTS has been reported in type 1 DM patients, where it may rise to 85% after 54 disease years<sup>[56]</sup>.

A case-control Italian study<sup>[57]</sup> reported that, not only overt DM, but also abnormal glucose metabolism was present in a high percentage of the subjects with idiopathic CTS. This finding led the authors to propose insulin resistance screening for all patients with CTS, as they found insulin resistance in 80% of patients: 45% had impaired glucose tolerance, 14% newly diagnosed DM and 20% insulin resistance only<sup>[57]</sup>.

The dominant hand is the most commonly affected in CTS, with a prevalence for females, where the tunnel tends to be smaller, and in obese DM patients<sup>[53,58]</sup>.

Such a strong association between MNW/CTS and DM is underpinned by the fact that DM nerves are very prone to compression due to metabolic and vascular factors occurring in a DM already in the prediabetic stage. Indeed, increased pressure in the carpal tunnel, which rises up to 8-10-fold in the flexion/extension movements of the wrist, and nerve traction may reduce the intra-neural microcirculation, damage the myelin sheath and the axonal function, as well as the connective structures, in a vicious circle where the nerve swelling, due to oedema and hypoxia, are a pivotal aggravating factor in the pathophysiology of CTS<sup>[59]</sup> (Figure 2).

Median nerve entrapment in the carpal tunnel with neural mobilization during anatomical stress may lead to conduction failure also in the non-diabetic population. This has recently been demonstrated by a study where recruitment properties of the median nerve were studied by the stimulus-response curve before and after intermittent-repetitive neural mobilization,

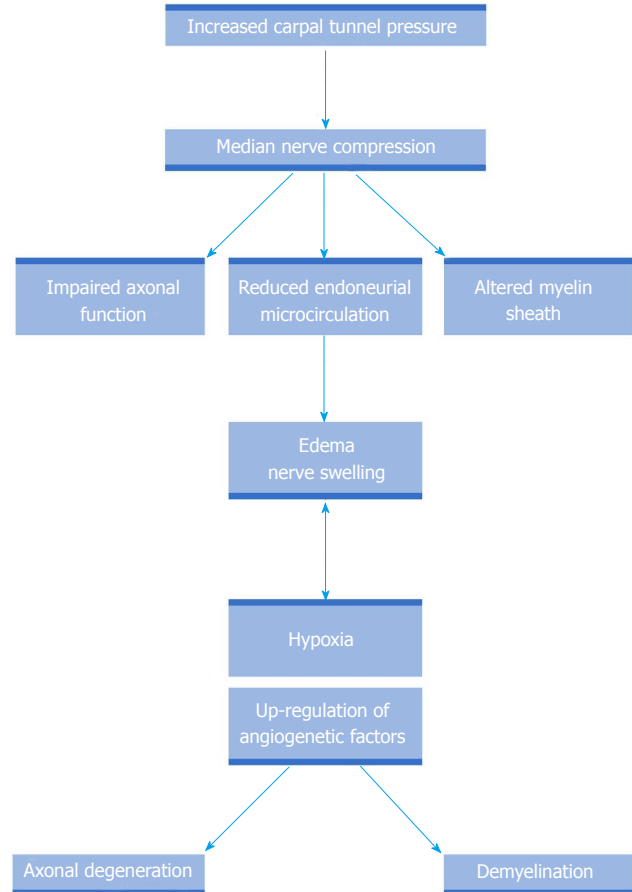


Figure 2 Carpal tunnel pathophysiology (modified from Aboonq<sup>[59]</sup>, 2015).

in subjects with and without CTS<sup>[60]</sup>. Only subjects with CTS exhibited a strikingly abnormal stimulus-response curve. This finding suggests that compressive forces may alter energy-dependent processes during anatomical stress in elongation, leading to conduction block in axons<sup>[60]</sup>. Taken as a whole, these findings do seem to imply that once an entrapment has occurred in MNW/CTS, anatomical stress may enhance the pre-existing metabolic and ischemic nerve damage within a sort of vicious circle, leading to axonal degeneration and to a worsening of the entrapment, unless surgery is performed.

When the severity of an electrophysiologically confirmed CTS was compared between patients with DM (and no concomitant metabolic syndrome) and patients with metabolic syndrome, it was observed to be more severe in those with a metabolic syndrome<sup>[61]</sup>. This finding suggests the presence of other disease modifying factors related to the metabolic syndrome.

Nerve conduction studies are the mainstay in the diagnostic approach to the MNW and CTS. Although quantitative sensory testing for the different modalities (temperature, pain, vibration perception threshold, perception testing) may be more sensitive than standard clinical tests<sup>[62]</sup>, it has considerable subjective components making it unreliable for diagnosis<sup>[15,63]</sup>.

Electrophysiological studies, that measure median nerve sensory and motor conduction parameters, not

only allow for a diagnosis of MNW, but also the staging of its severity, as they may detect focal abnormalities within the carpal tunnel itself. The techniques for an electrodiagnosis of CTS were described and assessed by Werner *et al.*<sup>[64]</sup>, in an American Association of Neuromuscular and Electrodiagnostic Medicine monograph. The sensory response is particularly useful to diagnose CTS, as sensory fibres have a larger proportion of large myelinated fibres, with a higher energy requirement, that are more susceptible to ischemic and metabolic damage. Comparison of the median nerve sensory latency with ulnar or radial latency has been demonstrated to be more effective than the use of absolute median nerve latency in documenting the median nerve entrapment within the carpal tunnel<sup>[64]</sup>.

An electrodiagnosis of CTS may be particularly challenging in DM patients with a polyneuropathy, where the comparative tests between the ulnar and radial nerve may necessitate that the values be adjusted from those used in the general population to more conservative cut-off values<sup>[64]</sup>. Moreover, segmental and comparative median nerve conduction tests (the disto-proximal latency ratio) showed a high sensitivity of 90% in DM patients affected by polyneuropathy, which is similar to that of the median-ulnar sensory latency to digit 4 comparison<sup>[65]</sup>.

The measures needed to achieve a minimum standard of care for the use of electrodiagnostic testing in the suspicion of a CTS have been defined according to the guidelines developed by the American Association of Neuromuscular and Electrodiagnostic Medicine<sup>[66]</sup>.

MNW is the entrapment neuropathy most frequently studied with ultrasonography. Enlargement of the median nerve cross-sectional area at the distal wrist crease is an accurate parameter for the diagnosis of CTS, with a sensitivity and specificity of > 85% in several studies<sup>[47]</sup>. The median nerve has a lower mobility in patients affected by CTS than healthy controls and this decreased range of movement can be quantified in both lateral and distal-proximal planes, according to set grading scales<sup>[47]</sup>. A recent study<sup>[67]</sup> suggests that ultrasonography be used to make a differential diagnosis between EN and diabetic neuropathy in DM patients. There was a wider median nerve cross-sectional area in DM patients with polyneuropathy than in controls, in particular in the CTS subgroup, where there was a larger cross-sectional area at the wrist and wrist-to-forearm<sup>[67]</sup>.

The treatment of CTS is mainly surgical, aimed at decompression of the median nerve by sectioning the carpal transverse ligament. Surgical nerve release can be done either by an open approach (OCTR) or endoscopically (ECTR). Although the two approaches differ only slightly as to pain relief and improvement of functional status, there may be a functionally significant benefit of ECTR over OCTR in improving grip strength<sup>[68]</sup>. Several non-surgical approaches, including oral steroids, splinting, ultrasound, yoga and carpal bone mobilization, have been suggested for patients with mild-moderate symptoms, with significant short-term benefit, although

long-term efficacy remains to be ascertained<sup>[69]</sup>. In the DM population, where the metabolic derangement may impair nerve regeneration<sup>[43]</sup>, the question arises whether the treatment, above all surgery, would maintain the same long-term efficacy as it does in the general population. Such a question has been addressed by some studies. The outcome of CTS surgical release was evaluated at one month and one year in DM patients and was observed to be inferior and slower than in non-diabetic controls<sup>[70]</sup>. Such a difference was attributed to metabolic factors and impaired nerve regeneration, which are likely to be responsible for the slower recovery in DM patients<sup>[70]</sup>. A worse surgical outcome was reported at 10 post-surgical years for DM patients with CTS, compared to idiopathic CTS patients<sup>[71]</sup>. However, another recent study was carried out on 35 patients and 31 normal controls with a 5-year follow-up. It reported that after surgical release of CTS, DM subjects had a long-term improvement in sensory, motor function and cold intolerance, comparable to that of non-diabetic controls<sup>[72]</sup>. Interestingly, the improvement in cold intolerance would be consistent with a potential for long-term regeneration of small fibers<sup>[72]</sup>. Thomsen *et al.*<sup>[73]</sup> also assessed the neurophysiologic recovery after carpal tunnel release in the same sample of DM patients, who had significantly impaired nerve conduction parameters, both before and after surgical carpal tunnel release, compared to non-diabetic patients. Nevertheless, neurophysiologic recovery after carpal tunnel surgery did not differ between the two patient groups or between diabetic patients, with or without peripheral neuropathy. Thus, relevant neurophysiologic impairment of the median nerve, or signs of peripheral neuropathy, is not likely to preclude significant recovery after carpal tunnel release in DM patients<sup>[73]</sup>. Even the quality of life, evaluated with generic and disease-specific questionnaires (SF-36 and Boston Carpal Tunnel Questionnaire, respectively), that was more impaired in DM CTS patients than in non-diabetic CTS subjects and the general population, had the same post-surgical scores for both DM and non-diabetic patients<sup>[74]</sup>. Therefore, the authors stated that DM patients should be given the same surgical option for CTS treatment as non-diabetic patients<sup>[73,74]</sup>.

## UNE AND ULNAR ENTRAPMENT NEUROPATHY AT THE WRIST (UNW)

The second most frequent entrapment neuropathy involves the UNE, in the retroepicondylar groove or under the humeroulnar aponeurotic arcade, *i.e.*, the cubital tunnel. A study by Mondelli *et al.*<sup>[75]</sup> investigated the UNE incidence in the general population, where there was a crude incidence of 24.7 per 100000 person-years and a standardized incidence of 20.9 per 100000 person-years. A prospective study<sup>[76]</sup> was carried out on a group of subjects with suspected UNE given electrodiagnostic and ultrasonographic examination.

It reported that the entrapment was localized in the retroepicondylar groove in 76% of the cases, where it was mainly demyelinating and at the humeroulnar aponeurotic arcade in 17%, where it was mostly axonal. UNE may occur without symptoms or be accompanied by painful paraesthesias in the fourth and/or fifth digit or by hypothenar or interosseus muscle weakness and wasting.

Clinical and neurophysiologic findings of a severe ulnar neuropathy were previously reported in a group of patients affected by DM with severe systemic complications<sup>[77]</sup>. Another four patients with type I DM and clinical findings suggestive of severe ulnar neuropathy have been described, where nerve conduction studies detected a partial conduction block or abnormal temporal dispersion within the forearm segment of the ulnar nerve, along with a mild underlying polyneuropathy<sup>[78]</sup>. The authors raised the question whether the UNE was due to an increased propensity to focal compression of the ulnar nerve within the humeroulnar arcade, or whether it represented a localized manifestation of the generalized polyneuropathy<sup>[78]</sup>. According to the advances made in DN pathophysiology, this seems to be only an apparent contradiction, in as much as, in reality, the metabolic derangement of diabetic nerves may underpin their remarkable liability to compression.

A more recent study<sup>[19]</sup> assessed the prevalence and electrophysiological features of ulnar entrapment neuropathy, according to the American Association of Electrodiagnostic Medicine protocol<sup>[79]</sup>. A cohort of 64 consecutive DM patients were studied and UNE was electrodiagnosed in 34% (18% were not polyneuropathic); UNW was detected in 11% of this sample<sup>[19]</sup>. On the basis of such a high proportion of patients (45%) with neurophysiological alterations consistent with ulnar EN at both sites (elbow and wrist), the authors concluded that the ulnar nerve, similarly to the median nerve, is very susceptible to focal entrapment in DM. Moreover, they suggested that upper limb sensory and motor NCS, including motor conduction velocity across the elbow, be routinely evaluated in the staging of DM patients<sup>[19]</sup>.

Furthermore, the frequent neurophysiological abnormalities detected on the ulnar nerve by this electrodiagnostic study<sup>[19]</sup> were mostly asymptomatic and only a small proportion of patients with a diagnosis of UNE showed the clinical signs of EN. This finding that UNE was mainly subclinical is in agreement with previous evidence related to MNW, which was asymptomatic in one third of DM patients<sup>[18,20,44,45]</sup>. It is also in line with the hypothesis that there is an alteration of the threshold of the sensory nerve fibres in DM and this may well explain the lower propensity for DN patients to develop a clinical symptomatology<sup>[18,20,44,45]</sup>.

Furthermore, in the same study<sup>[19]</sup>, UNW was concomitant with MNW in all but one case. Indeed, the question arises as to the association between UNW and MNW, where the discrepancy in literature is most likely to be due to the different methods adopted<sup>[80]</sup>.

Indeed, in DM patients, where the frequency of MNW is high, concomitant involvement of Guyon's canal (UNW) has been shown to reduce the sensitivity of the median-ulnar comparative studies<sup>[81]</sup>. Therefore, the neurophysiological diagnosis of concomitant CTS and UNW may present a challenge, above all in DN patients. A retrospective case-control study<sup>[82]</sup>, carried out on an electrodiagnostic database, included 1924 patients evaluated for CTS and 1024 DM patients investigated for CTS and/or polyneuropathy. A logistic regression analysis showed that the presence of CTS was associated with a two-fold risk of UNW in both idiopathic CTS and DM CTS groups. These findings suggest that the presence of concomitant UNW and CTS should be carefully pursued in nerve conduction studies, above all in DM patients.

A study by Mondelli *et al.*<sup>[75]</sup> compared the prevalence of DM in two consecutive samples of patients with UNE and CTS and reported that it was remarkably similar, *i.e.*, 6.0% and 6.6% respectively. Indeed, patients with UNE and DM were clinically and neurophysiologically indistinguishable from other UNE patients (both idiopathic and post-traumatic). The only difference was a smaller amplitude of the sensory response in the DM patients, which may well be attributable to the underlying axonal polyneuropathy<sup>[75]</sup>. These findings strengthen the similarities between median and ulnar EN in DM, which obviously act in the same way on peripheral nerves at both the upper and lower limbs, predisposing them to compression in anatomically narrow sites, where the nerves are exposed to increased pressure and repetitive strain.

## EN AT THE LOWER LIMBS

It seems that entrapments of the ulnar and median nerve are not only a typical electrophysiological feature of polyneuropathy in DM, but also the early subclinical sign of peripheral nerve damage, even when a generalized diabetic neuropathy is not yet evident. Whilst EN at the lower limbs seem to be less frequent feature of DM. Indeed, the evidence of an increased frequency of common peroneal nerve entrapment in DM at the level of the fibular head and of the tarsal tunnel syndrome is less overwhelming, compared to upper limb focal neuropathies.

In the past it was reported that DM was the underlying cause of peroneal neuropathy in only 5%-12% of patients<sup>[83]</sup>. However, a more recent study was carried out to determine whether peripheral neuropathy could explain the apparent association between DM and disability in ageing subjects. It reported that reduced peroneal motor response amplitude at multiple sites and weakness of foot dorsiflexion were found in two thirds of the sample of DN patients over 65<sup>[15,84]</sup>.

A similar impairment of peroneal nerve conduction parameters was observed in subjects 65 years or older in a study<sup>[85]</sup>, carried out to determine whether DM was associated with objective measures of physical and



peripheral function. It concluded that DM patients had a decreased conduction velocity and motor response amplitude at the lower limbs, along with a reduced walking speed, compared to the non-diabetic subjects<sup>[85]</sup>. However, if direct neurophysiological signs of entrapment, *e.g.*, conduction block or reduced motor conduction velocity across the fibular head, are not carefully searched for and detected, peroneal axonal damage may be consequent to DN itself or to an L5 radiculopathy or a lumbar spinal stenosis. These conditions may even be superimposed on DN, making for a complex differential diagnosis.

The diagnosis of tarsal tunnel syndrome is even more challenging, as it is characterized by entrapment of the tibialis nerve as it curves behind the medial malleolus underneath the flexor retinaculum. There may be a selective or prevailing entrapment of the medial or lateral plantar nerves, two of the terminal branches of tibial nerve, in a tarsal tunnel syndrome. Indeed, this is more difficult to demonstrate without the adoption of a complex electrodiagnostic protocol with segmental analysis of the motor conduction velocity in the distal tracts of the tibial nerve<sup>[86]</sup>. Such shortcomings in neurophysiological investigation protocols seem to be common to several studies on neuropathy at the lower limbs in DM, making them unreliable when investigating entrapment. Therefore, these methodological limits could be considered a plausible explanation for the less detailed evidence on entrapment in the lower limbs, than what is available for the upper extremities in the general population and even more so in DM patients. Indeed, similarities between CTS and tarsal tunnel syndrome might be expected as they have a common pathophysiological background that predisposes the nerves to external compression.

Surgical nerve release seems to find a rationale in the "revisited" Upton and McComas's "double crush" hypothesis (Figure 1)<sup>[15,34]</sup>, where DN (with nerve swelling) represents the "first crush" and nerve compression at the tarsal tunnel or peroneal head the "second crush"<sup>[87]</sup>, despite the often limited electrodiagnostic evidence for entrapment superimposed to the length-dependent DN. This hypothesis has received recent support by nerve ultrasonography that demonstrated an increased cross-sectional area in nerves affected by neuropathy<sup>[47]</sup>. Moreover, ultrasound imaging was used to quantify the magnitude and timing of tibial nerve excursion during ankle dorsiflexion in patients with DM and was compared to matched healthy controls<sup>[88]</sup>. The results showed that the nerve cross-sectional area was increased at the ankle in the DM group, where the tibial nerve longitudinal excursion at the ankle and knee was reduced proportionally to the severity of neuropathy. Moreover, on the basis that a larger tibial nerve size within the tarsal tunnel in patients with DM may restrict longitudinal excursion, it has been hypothesized that such altered tibial nerve biomechanics may be related to painful symptoms during functional activities<sup>[88]</sup>.

### **Surgical decompression of nerves at the lower limbs**

Several studies have been based on the "double crush" hypothesis (Figure 1), from the pioneering work by Dellon<sup>[89]</sup> to more recent studies<sup>[90,91]</sup>, which evaluated the efficacy of surgical decompression in DN patients. Considering Valdivia Valdivia *et al.*<sup>[90]</sup>'s retrospective review, the results of neurolysis on multiple sites of chronic nerve compression in the lower extremity were analyzed in 158 consecutive patients, 96 with DM and 62 with idiopathic neuropathy. A significant post-operative improvement was reported in sensation and balance at a minimum follow-up of 1 year; even pain improved, as demonstrated by a decrease in the Visual Analogic Scale score. There was no statically significant difference in outcomes between patients with DM vs idiopathic neuropathy in response to nerve decompression<sup>[90]</sup>. Another study by Liao *et al.*<sup>[91]</sup> investigated into the effect surgical decompression had on painful DN as to the pain distribution, where a total of 306 patients, with painful diabetic lower-extremity neuropathy were treated with Dellon surgical nerve decompression. Patients had pre- and post-surgical (were appropriate) clinical evaluation and high-resolution ultrasonography (cross-sectional area), as well as nerve conduction studies (tibial and common peroneal nerve conduction velocity). Surgical patients were retrospectively assigned into two subgroups, *i.e.*, focal and diffuse pain, according to the distribution of the diabetic neuropathic pain. The control group included 92 non-surgical patients with painful DN. After surgical decompression, the surgical group had a higher reduction in pain (measured as Visual Analogic Scale score) and an improvement in nerve conduction and cross-sectional area than did the control group. As was expected, based on the rationale on the surgical decompression approach, a greater improvement in Visual Analogic Scale and cross-sectional area was observed in the focal pain group than in the diffuse pain group. The authors concluded that decompression of multiple lower-extremity peripheral nerves was effective in patients with painful DN to a greater extent in patients with focal symptoms<sup>[91]</sup>.

However, unfortunately, these two studies show relevant methodological shortcomings. Firstly, there was no demonstration of a precise site of entrapment by direct electrodiagnostic signs along nerves, which showed only axonal damage subsequent to DN. Furthermore, serial measurements of nerve motor conduction velocities may show a variability<sup>[92]</sup> which was not taken into account in the post-surgical evaluation of the improved conduction velocity along tibial and common peroneal nerves. In addition, most of the outcome measures evaluated by these studies are subjective, making the definition of focal pain in the study of Liao *et al.*<sup>[91]</sup>, 2014 questionable. Therefore, we are of the opinion that further neurophysiological studies should be carried out in an effort to better characterize EN superimposed on DN at the lower limbs. Moreover, further prospective studies, based on detailed

electrodiagnostic and ultrasonographic protocols aimed at localizing the sites of nerve compression are welcome to better assess the efficacy of surgical nerve decompression in patients suffering from painful DN.

## CONCLUSION

EN are so common in DM, at any stage, that they may be considered a neurophysiological hallmark of peripheral nerve involvement in DM. Indeed, EN, particularly in the upper limbs, may represent the earliest neurophysiological abnormalities, which are often asymptomatic, even in the absence of a generalized polyneuropathy or, usually later in the natural history of DM, they may be superimposed on a generalized DN.

The remarkable frequency of EN in DM is underpinned by a peculiar pathophysiological background. The peripheral nerves, due to the metabolic alterations consequent to altered glucose metabolism, even in the preclinical stage, show both functional impairment and structural changes, mainly swelling, which makes them more prone to entrapment in anatomically constrained channels. The diagnosis of EN relies mainly on nerve conduction studies and may sometimes be challenging, mostly in DM patients with a generalized polyneuropathy. Despite this, we believe that an EN diagnosis is a must, not only for the staging of DM, but also due to the fact that the treatment of choice for numerous EN cases may have to be surgical.

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## REFERENCES

- 1 **Tesfaye S**, Boulton AJ, Dyck PJ, Freeman R, Horowitz M, Kempler P, Lauria G, Malik RA, Spallone V, Vinik A, Bernardi L, Valensi P. Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. *Diabetes Care* 2010; **33**: 2285-2293 [PMID: 20876709 DOI: 10.2337/dc10-1303]
- 2 **Dyck PJ**. Detection, characterization, and staging of polyneuropathy: assessed in diabetics. *Muscle Nerve* 1988; **11**: 21-32 [PMID: 3277049 DOI: 10.1002/mus.880110106]
- 3 **Vinik AI**, Park TS, Stansberry KB, Pittenger GL. Diabetic neuropathies. *Diabetologia* 2000; **43**: 957-973 [PMID: 10990072 DOI: 10.1007/s001250051477]
- 4 **Tesfaye S**, Stevens LK, Stephenson JM, Fuller JH, Plater M, Ionescu-Tirgoviste C, Nuber A, Pozza G, Ward JD. Prevalence of diabetic peripheral neuropathy and its relation to glycaemic control and potential risk factors: the EURODIAB IDDM Complications Study. *Diabetologia* 1996; **39**: 1377-1384 [PMID: 8933008 DOI: 10.1007/s001250050586]
- 5 **Said G**. Focal and multifocal diabetic neuropathies. *Arq Neuropsiquiatr* 2007; **65**: 1272-1278 [PMID: 18345446 DOI: 10.1590/S0004-282X2007000700037]
- 6 **Dyck PJ**, Albers JW, Andersen H, Arezzo JC, Biessels GJ, Bril V, Feldman EL, Litchy WJ, O'Brien PC, Russell JW; Toronto Expert Panel on Diabetic Neuropathy. Diabetic polyneuropathies: update on research definition, diagnostic criteria and estimation of severity. *Diabetes Metab Res Rev* 2011; **27**: 620-628 [PMID: 21695763 DOI: 10.1002/dmrr.1226]
- 7 **Thomas PK**. Classification, differential diagnosis, and staging of diabetic peripheral neuropathy. *Diabetes* 1997; **46** Suppl 2: S54-S57 [PMID: 9285500 DOI: 10.2337/diab.46.2.S54]
- 8 **Boulton AJ**, Vinik AI, Arezzo JC, Bril V, Feldman EL, Freeman R, Malik RA, Maser RE, Sosenko JM, Ziegler D. Diabetic neuropathies: a statement by the American Diabetes Association. *Diabetes Care* 2005; **28**: 956-962 [PMID: 15793206 DOI: 10.2337/diacare.28.4.956]
- 9 **Dyck PJ**, Karnes JL, O'Brien PC, Litchy WJ, Low PA, Melton LJ. The Rochester Diabetic Neuropathy Study: reassessment of tests and criteria for diagnosis and staged severity. *Neurology* 1992; **42**: 1164-1170 [PMID: 1603343 DOI: 10.1212/WNL.42.6.1164]
- 10 **Archer AG**, Watkins PJ, Thomas PK, Sharma AK, Payan J. The natural history of acute painful neuropathy in diabetes mellitus. *J Neurol Neurosurg Psychiatry* 1983; **46**: 491-499 [PMID: 6875582 DOI: 10.1136/jnnp.46.6.491]
- 11 **Raff MC**, Asbury AK. Ischemic mononeuropathy and mononeuropathy multiplex in diabetes mellitus. *N Engl J Med* 1968; **279**: 17-21 [PMID: 4297979 DOI: 10.1056/NEJM196807042790104]
- 12 **Chokroverty S**, Reyes MG, Rubino FA, Tonaki H. The syndrome of diabetic amyotrophy. *Ann Neurol* 1977; **2**: 181-194 [PMID: 215072 DOI: 10.1002/ana.410020303]
- 13 **Llewellyn JG**, Thomas PK, King RH. Epineurial microvasculitis in proximal diabetic neuropathy. *J Neurol* 1998; **245**: 159-165 [PMID: 9553846 DOI: 10.1007/s004150050197]
- 14 **Asbury AK**, Aldredge H, Hershberg R, Fisher CM. Oculomotor palsy in diabetes mellitus: a clinico-pathological study. *Brain* 1970; **93**: 555-566 [PMID: 5507015 DOI: 10.1093/brain/93.3.555]
- 15 **Vinik A**, Mehrabyan A, Colen L, Boulton A. Focal entrapment neuropathies in diabetes. *Diabetes Care* 2004; **27**: 1783-1788 [PMID: 15220266 DOI: 10.2337/diacare.27.7.1783]
- 16 **Knopp M**, Rajabally YA. Common and less common peripheral nerve disorders associated with diabetes. *Curr Diabetes Rev* 2012; **8**: 229-236 [PMID: 22283678 DOI: 10.2174/157339912800564034]
- 17 **Stamboulis E**, Vassilopoulos D, Kalfakis N. Symptomatic focal mononeuropathies in diabetic patients: increased or not? *J Neurol* 2005; **252**: 448-452 [PMID: 15726259 DOI: 10.1007/s00415-005-0672-8]
- 18 **Rota E**, Quadri R, Fanti E, Isoardo G, Poglio F, Tavella A, Paolasso I, Ciaramitaro P, Bergamasco B, Cocito D. Electrophysiological findings of peripheral neuropathy in newly diagnosed type II diabetes mellitus. *J Peripher Nerv Syst* 2005; **10**: 348-353 [PMID: 16279983 DOI: 10.1111/j.1085-9489.2005.00046.x]
- 19 **Rota E**, Zavaroni D, Parietti L, Iafelice I, De Mitri P, Terlizzi E, Morelli N, Immovilli P, Guidetti D. Ulnar entrapment neuropathy in patients with type 2 diabetes mellitus: an electrodiagnostic study. *Diabetes Res Clin Pract* 2014; **104**: 73-78 [PMID: 24565211 DOI: 10.1016/j.diabres.2014.01.024]
- 20 **Albers JW**, Brown MB, Sima AA, Greene DA. Frequency of median mononeuropathy in patients with mild diabetic neuropathy in the early diabetes intervention trial (EDIT). Tolrestat Study Group For Edit (Early Diabetes Intervention Trial) *Muscle Nerve* 1996; **19**: 140-146 [PMID: 8559161]
- 21 **Perkins BA**, Bril V. Diabetic neuropathy: a review emphasizing diagnostic methods. *Clin Neurophysiol* 2003; **114**: 1167-1175 [PMID: 12842711 DOI: 10.1016/S1388-2457(03)00025-7]
- 22 **Dellon AL**, Mackinnon SE, Seiler WA. Susceptibility of the diabetic nerve to chronic compression. *Ann Plast Surg* 1988; **20**: 117-119 [PMID: 3355055 DOI: 10.1097/0000637-198802000-00004]
- 23 **Novella SP**, Inzucchi SE, Goldstein JM. The frequency of undiagnosed diabetes and impaired glucose tolerance in patients with idiopathic sensory neuropathy. *Muscle Nerve* 2001; **24**: 1229-1231 [PMID: 11494278 DOI: 10.1002/mus.1137]
- 24 **Singleton JR**, Smith AG, Bromberg MB. Increased prevalence of impaired glucose tolerance in patients with painful sensory neuropathy. *Diabetes Care* 2001; **24**: 1448-1453 [PMID: 11473085 DOI: 10.2337/diacare.24.8.1448]
- 25 **Sumner CJ**, Sheth S, Griffin JW, Cornblath DR, Polydefkis M. The spectrum of neuropathy in diabetes and impaired glucose tolerance. *Neurology* 2003; **60**: 108-111 [PMID: 12525727 DOI: 10.1212/WNL.60.1.108]

- 26 **Murakawa Y**, Zhang W, Pierson CR, Brismar T, Ostenson CG, Efendic S, Sima AA. Impaired glucose tolerance and insulinopenia in the GK-rat causes peripheral neuropathy. *Diabetes Metab Res Rev* 2002; **18**: 473-483 [PMID: 12469361 DOI: 10.1002/dmrr.326]
- 27 **Guo G**, Kan M, Martinez JA, Zochodne DW. Local insulin and the rapid regrowth of diabetic epidermal axons. *Neurobiol Dis* 2011; **43**: 414-421 [PMID: 21530660 DOI: 10.1016/j.nbd.2011.04.012]
- 28 **Zochodne DW**. Diabetes and the plasticity of sensory neurons. *Neurosci Lett* 2015; **596**: 60-65 [PMID: 25445357 DOI: 10.1016/j.neulet.2014.11.017]
- 29 **Sessions J**, Nickerson DS. Biologic Basis of Nerve Decompression Surgery for Focal Entrapments in Diabetic Peripheral Neuropathy. *J Diabetes Sci Technol* 2014; **8**: 412-418 [PMID: 24876595 DOI: 10.1177/1932296814525030]
- 30 **Misawa S**, Kuwabara S, Ogawara K, Kitano Y, Yagui K, Hattori T. Hyperglycemia alters refractory periods in human diabetic neuropathy. *Clin Neurophysiol* 2004; **115**: 2525-2529 [PMID: 15465442 DOI: 10.1016/j.clinph.2004.06.008]
- 31 **Song Z**, Fu DT, Chan YS, Leung S, Chung SS, Chung SK. Transgenic mice overexpressing aldose reductase in Schwann cells show more severe nerve conduction velocity deficit and oxidative stress under hyperglycemic stress. *Mol Cell Neurosci* 2003; **23**: 638-647 [PMID: 12932443 DOI: 10.1016/S1044-7431(03)00096-4]
- 32 **Nishimura T**, Hirata H, Tsujii M, Iida R, Hoki Y, Iino T, Ogawa S, Uchida A. Pathomechanism of entrapment neuropathy in diabetic and nondiabetic rats reared in wire cages. *Histol Histopathol* 2008; **23**: 157-166 [PMID: 17999372]
- 33 **Nemoto K**, Matsumoto N, Tazaki K, Horiuchi Y, Uchinishi K, Mori Y. An experimental study on the "double crush" hypothesis. *J Hand Surg Am* 1987; **12**: 552-559 [PMID: 3611653 DOI: 10.1016/S0363-5023(87)80207-1]
- 34 **Upton AR**, McComas AJ. The double crush in nerve entrapment syndromes. *Lancet* 1973; **2**: 359-362 [PMID: 4124532 DOI: 10.1016/S0140-6736(73)93196-6]
- 35 **Zahir KS**, Zahir FS, Thomas JG, Dudrick SJ. The double-crush phenomenon—an unusual presentation and literature review. *Conn Med* 1999; **63**: 535-538 [PMID: 10531704]
- 36 **Chowdhury SK**, Smith DR, Fernyhough P. The role of aberrant mitochondrial bioenergetics in diabetic neuropathy. *Neurobiol Dis* 2013; **51**: 56-65 [PMID: 22446165 DOI: 10.1016/j.nbd.2012.03.016]
- 37 **Valls-Canals J**, Povedano M, Montero J, Pradas J. Diabetic polyneuropathy. Axonal or demyelinating? *Electromyogr Clin Neurophysiol* 2002; **42**: 3-6 [PMID: 11851006]
- 38 **Cappellari A**, Airaghi L, Capra R, Ciammola A, Branchi A, Levi Minzi G, Bresolin N. Early peripheral nerve abnormalities in impaired glucose tolerance. *Electromyogr Clin Neurophysiol* 2005; **45**: 241-244 [PMID: 16083148]
- 39 **Bromberg MB**, Albers JW. Patterns of sensory nerve conduction abnormalities in demyelinating and axonal peripheral nerve disorders. *Muscle Nerve* 1993; **16**: 262-266 [PMID: 8383290 DOI: 10.1002/mus.880160304]
- 40 **Mackinnon SE**, Dellon AL, Hudson AR, Hunter DA. Chronic human nerve compression—a histological assessment. *Neuropathol Appl Neurobiol* 1986; **12**: 547-565 [PMID: 3561691]
- 41 **Tapadia M**, Mozaffar T, Gupta R. Compressive neuropathies of the upper extremity: update on pathophysiology, classification, and electrodiagnostic findings. *J Hand Surg Am* 2010; **35**: 668-677 [PMID: 20223605 DOI: 10.1016/j.jhsa.2010.01.007]
- 42 **Perkins BA**, Olaleye D, Bril V. Carpal tunnel syndrome in patients with diabetic polyneuropathy. *Diabetes Care* 2002; **25**: 565-569 [PMID: 11874948]
- 43 **Kennedy JM**, Zochodne DW. Impaired peripheral nerve regeneration in diabetes mellitus. *J Peripher Nerv Syst* 2005; **10**: 144-157 [PMID: 15958126 DOI: 10.1111/j.1085-9489.2005.0010205.x]
- 44 **Celiker R**, Basgöze O, Bayraktar M. Early detection of neurological involvement in diabetes mellitus. *Electromyogr Clin Neurophysiol* 1996; **36**: 29-35 [PMID: 8654318]
- 45 **Kim WK**, Kwon SH, Lee SH, Sunwoo IN. Asymptomatic electrophysiologic carpal tunnel syndrome in diabetics: entrapment or polyneuropathy. *Yonsei Med J* 2000; **41**: 123-127 [PMID: 10731930 DOI: 10.3349/ymj.2000.41.1.123]
- 46 **Ali ZS**, Pisapia JM, Ma TS, Zager EL, Heuer GG, Khoury V. Ultrasonographic Evaluation of Peripheral Nerves. *World Neurosurg* 2016; **85**: 333-339 [PMID: 26463397 DOI: 10.1016/j.wneu.2015.10.005]
- 47 **Cartwright MS**, Walker FO. Neuromuscular ultrasound in common entrapment neuropathies. *Muscle Nerve* 2013; **48**: 696-704 [PMID: 23681885 DOI: 10.1002/mus.23900]
- 48 **Pitarokoli K**, Kerasnoudis A, Behrendt V, Labedi A, Ayzenberg I, Gold R, Yoon MS. Facing the diagnostic challenge: Nerve ultrasound in diabetic patients with neuropathic symptoms. *Muscle Nerve* 2016; **54**: 18-24 [PMID: 26575030 DOI: 10.1002/mus.24981]
- 49 **Thakkar RS**, Del Grande F, Thawait GK, Andreisek G, Carrino JA, Chhabra A. Spectrum of high-resolution MRI findings in diabetic neuropathy. *AJR Am J Roentgenol* 2012; **199**: 407-412 [PMID: 22826404 DOI: 10.2214/AJR.11.7893]
- 50 **Padua L**, Padua R, Lo Monaco M, Aprile I, Tonali P. Multiperspective assessment of carpal tunnel syndrome: a multicenter study. Italian CTS Study Group. *Neurology* 1999; **53**: 1654-1659 [PMID: 10563608]
- 51 **Mediouni Z**, Bodin J, Dale AM, Herquelot E, Carton M, Leclerc A, Fouquet N, Dumontier C, Roquelaure Y, Evanoff BA, Descatha A. Carpal tunnel syndrome and computer exposure at work in two large complementary cohorts. *BMJ Open* 2015; **5**: e008156 [PMID: 26353869 DOI: 10.1136/bmjopen-2015-008156]
- 52 **Makepeace A**, Davis WA, Bruce DG, Davis TM. Incidence and determinants of carpal tunnel decompression surgery in type 2 diabetes: the Fremantle Diabetes Study. *Diabetes Care* 2008; **31**: 498-500 [PMID: 18070996 DOI: 10.2337/dc07-2058]
- 53 **Awada AA**, Bashi SA, Aljumah MA, Heffernan LP. Carpal Tunnel Syndrome in type 2 diabetic patients. *Neurosciences (Riyadh)* 2000; **5**: 219-222 [PMID: 24276599]
- 54 **Chen LH**, Li CY, Kuo LC, Wang LY, Kuo KN, Jou IM, Hou WH. Risk of Hand Syndromes in Patients With Diabetes Mellitus: A Population-Based Cohort Study in Taiwan. *Medicine (Baltimore)* 2015; **94**: e1575 [PMID: 26469895 DOI: 10.1097/MD.0000000000001575]
- 55 **van Dijk MA**, Reitsma JB, Fischer JC, Sanders GT. Indications for requesting laboratory tests for concurrent diseases in patients with carpal tunnel syndrome: a systematic review. *Clin Chem* 2003; **49**: 1437-1444 [PMID: 12928223]
- 56 **Singh R**, Gamble G, Cundy T. Lifetime risk of symptomatic carpal tunnel syndrome in Type 1 diabetes. *Diabet Med* 2005; **22**: 625-630 [PMID: 15842519 DOI: 10.1111/j.1464-5491.2005.01487.x]
- 57 **Plastino M**, Fava A, Carmela C, De Bartolo M, Ermo C, Cristiano D, Ettore M, Abenavoli L, Bosco D. Insulin resistance increases risk of carpal tunnel syndrome: a case-control study. *J Peripher Nerv Syst* 2011; **16**: 186-190 [PMID: 22003933 DOI: 10.1111/j.1529-8027.2011.00344.x]
- 58 **Becker J**, Nora DB, Gomes I, Stringari FF, Seitensius R, Panosso JS, Ehlers JC. An evaluation of gender, obesity, age and diabetes mellitus as risk factors for carpal tunnel syndrome. *Clin Neurophysiol* 2002; **113**: 1429-1434 [PMID: 12169324 DOI: 10.1016/S1388-2457(02)00201-8]
- 59 **Aboonq MS**. Pathophysiology of carpal tunnel syndrome. *Neurosciences (Riyadh)* 2015; **20**: 4-9 [PMID: 25630774]
- 60 **Ginanneschi F**, Cioncoloni D, Bigliazzi J, Bonifazi M, Lorè C, Rossi A. Sensory axons excitability changes in carpal tunnel syndrome after neural mobilization. *Neurol Sci* 2015; **36**: 1611-1615 [PMID: 25896622 DOI: 10.1007/s10072-015-2218-x]
- 61 **Gül Yurdakul F**, Bodur H, Özpınar Çakmak Ö, Ateş C, Sivas F, Eser F, Yılmaz Taşdelen Ö. On the Severity of Carpal Tunnel Syndrome: Diabetes or Metabolic Syndrome. *J Clin Neurol* 2015; **11**: 234-240 [PMID: 26174786 DOI: 10.3988/jcn.2015.11.3.234]
- 62 **Siemionow M**, Zielinski M, Sari A. Comparison of clinical evaluation and neurosensory testing in the early diagnosis of superimposed entrapment neuropathy in diabetic patients. *Ann Plast Surg* 2006; **57**: 41-49 [PMID: 16799307 DOI: 10.1097/01.sap.0000210634.98344.47]
- 63 **Werner RA**, Andary M. Carpal tunnel syndrome: pathophysiology and clinical neurophysiology. *Clin Neurophysiol* 2002; **113**: 1373-1381 [PMID: 12169318]



- 64 **Werner RA**, Andary M. Electrodiagnostic evaluation of carpal tunnel syndrome. *Muscle Nerve* 2011; **44**: 597-607 [PMID: 21922474 DOI: 10.1002/mus.22208]
- 65 **Gazioglu S**, Boz C, Cakmak VA. Electrodiagnosis of carpal tunnel syndrome in patients with diabetic polyneuropathy. *Clin Neurophysiol* 2011; **122**: 1463-1469 [PMID: 21330198 DOI: 10.1016/j.clinph.2010.11.021]
- 66 **Sandin KJ**, Asch SM, Jablonski CK, Kilmer DD, Nuckols TK. Clinical quality measures for electrodiagnosis in suspected carpal tunnel syndrome. *Muscle Nerve* 2010; **41**: 444-452 [PMID: 20336661 DOI: 10.1002/mus.21617]
- 67 **Moon HI**, Kwon HK, Kim L, Lee HJ, Lee HJ. Ultrasonography of palm to elbow segment of median nerve in different degrees of diabetic polyneuropathy. *Clin Neurophysiol* 2014; **125**: 844-848 [PMID: 24269093 DOI: 10.1016/j.clinph.2013.10.041]
- 68 **Vasiliadis HS**, Georgoulas P, Shrier I, Salanti G, Scholten RJ. Endoscopic release for carpal tunnel syndrome. *Cochrane Database Syst Rev* 2014; **(1)**: CD008265 [PMID: 24482073 DOI: 10.1002/14651858.CD008265.pub2]
- 69 **O'Connor D**, Marshall S, Massy-Westropp N. Non-surgical treatment (other than steroid injection) for carpal tunnel syndrome. *Cochrane Database Syst Rev* 2003; **(1)**: CD003219 [PMID: 12535461 DOI: 10.1002/14651858.CD003219]
- 70 **Ozkul Y**, Sabuncu T, Kocabey Y, Nazligul Y. Outcomes of carpal tunnel release in diabetic and non-diabetic patients. *Acta Neurol Scand* 2002; **106**: 168-172 [PMID: 12174177]
- 71 **Gulabi D**, Cecen G, Guclu B, Cecen A. Carpal tunnel release in patients with diabetes result in poorer outcome in long-term study. *Eur J Orthop Surg Traumatol* 2014; **24**: 1181-1184 [PMID: 24442385 DOI: 10.1007/s00590-014-1418-z]
- 72 **Thomsen NO**, Cederlund RI, Andersson GS, Rosén I, Björk J, Dahlin LB. Carpal tunnel release in patients with diabetes: a 5-year follow-up with matched controls. *J Hand Surg Am* 2014; **39**: 713-720 [PMID: 24582843 DOI: 10.1016/j.jhsa.2014.01.012]
- 73 **Thomsen NO**, Rosén I, Dahlin LB. Neurophysiologic recovery after carpal tunnel release in diabetic patients. *Clin Neurophysiol* 2010; **121**: 1569-1573 [PMID: 20413347 DOI: 10.1016/j.clinph.2010.03.014]
- 74 **Thomsen NO**, Cederlund R, Björk J, Dahlin LB. Health-related quality of life in diabetic patients with carpal tunnel syndrome. *Diabet Med* 2010; **27**: 466-472 [PMID: 20536520 DOI: 10.1111/j.1464-5491.2010.02970.x]
- 75 **Mondelli M**, Giannini F, Ballerini M, Ginanneschi F, Martorelli E. Incidence of ulnar neuropathy at the elbow in the province of Siena (Italy). *J Neurol Sci* 2005; **234**: 5-10 [PMID: 15993135 DOI: 10.1016/j.jns.2005.02.010]
- 76 **Omejec G**, Podnar S. Precise localization of ulnar neuropathy at the elbow. *Clin Neurophysiol* 2015; **126**: 2390-2396 [PMID: 25743266 DOI: 10.1016/j.clinph.2015.01.023]
- 77 **Schady W**, Abuaisha B, Boulton AJ. Observations on severe ulnar neuropathy in diabetes. *J Diabetes Complications* 1998; **12**: 128-132 [PMID: 9618067]
- 78 **Acosta JA**, Hoffman SN, Raynor EM, Nardin RA, Rutkove SB. Ulnar neuropathy in the forearm: A possible complication of diabetes mellitus. *Muscle Nerve* 2003; **28**: 40-45 [PMID: 12811771 DOI: 10.1002/mus.10387]
- 79 **Campbell WW**. Guidelines in electrodiagnostic medicine. Practice parameter for electrodiagnostic studies in ulnar neuropathy at the elbow. *Muscle Nerve Suppl* 1999; **8**: S171-S205 [PMID: 16921634]
- 80 **Moghtaderi A**, Ghafarpour M. The dilemma of ulnar nerve entrapment at wrist in carpal tunnel syndrome. *Clin Neurol Neurosurg* 2009; **111**: 151-155 [PMID: 19084328 DOI: 10.1016/j.clineuro.2008.09.012]
- 81 **Imada M**, Misawa S, Sawai S, Tamura N, Kanai K, Sakurai K, Sakamoto S, Nomura F, Hattori T, Kuwabara S. Median-radial sensory nerve comparative studies in the detection of median neuropathy at the wrist in diabetic patients. *Clin Neurophysiol* 2007; **118**: 1405-1409 [PMID: 17452013 DOI: 10.1016/j.clinph.2007.03.003]
- 82 **Kiyiloglu N**, Akyildiz UO, Ozkul A, Akyol A. Carpal tunnel syndrome and ulnar neuropathy at the wrist: comorbid disease or not? *J Clin Neurophysiol* 2011; **28**: 520-523 [PMID: 21946366 DOI: 10.1097/WNP.0b013e318231c2cc]
- 83 **Garland H**, Moorhouse D. Compressive lesions of the external popliteal (common peroneal) nerve. *Br Med J* 1952; **2**: 1373-1378 [PMID: 12997789]
- 84 **Resnick HE**, Stansberry KB, Harris TB, Tirivedi M, Smith K, Morgan P, Vinik AI. Diabetes, peripheral neuropathy, and old age disability. *Muscle Nerve* 2002; **25**: 43-50 [PMID: 11754184]
- 85 **Chiles NS**, Phillips CL, Volpato S, Bandinelli S, Ferrucci L, Guralnik JM, Patel KV. Diabetes, peripheral neuropathy, and lower-extremity function. *J Diabetes Complications* 2014; **28**: 91-95 [PMID: 24120281 DOI: 10.1016/j.jdiacomp.2013.08.007]
- 86 **Troni W**, Parino E, Pisani PC, Pisani G. Segmental analysis of motor conduction velocity in distal tracts of tibial nerve: a coaxial needle electrode study. *Clin Neurophysiol* 2010; **121**: 221-227 [PMID: 19948425 DOI: 10.1016/j.clinph.2009.10.005]
- 87 **Dellon AL**. Diabetic neuropathy: review of a surgical approach to restore sensation, relieve pain, and prevent ulceration and amputation. *Foot Ankle Int* 2004; **25**: 749-755 [PMID: 15566708]
- 88 **Boyd BS**, Dilley A. Altered tibial nerve biomechanics in patients with diabetes mellitus. *Muscle Nerve* 2014; **50**: 216-223 [PMID: 24375463 DOI: 10.1002/mus.24155]
- 89 **Dellon AL**. Treatment of symptomatic diabetic neuropathy by surgical decompression of multiple peripheral nerves. *Plast Reconstr Surg* 1992; **89**: 689-697; discussion 698-699 [PMID: 1546082]
- 90 **Valdivia Valdivia JM**, Weinand M, Maloney CT, Blount AL, Dellon AL. Surgical treatment of superimposed, lower extremity, peripheral nerve entrapments with diabetic and idiopathic neuropathy. *Ann Plast Surg* 2013; **70**: 675-679 [PMID: 23673565 DOI: 10.1097/SAP.0b013e3182764fb0]
- 91 **Liao C**, Zhang W, Yang M, Ma Q, Li G, Zhong W. Surgical decompression of painful diabetic peripheral neuropathy: the role of pain distribution. *PLoS One* 2014; **9**: e109827 [PMID: 25290338 DOI: 10.1371/journal.pone.0109827]
- 92 **Bleasel AF**, Tuck RR. Variability of repeated nerve conduction studies. *Electroencephalogr Clin Neurophysiol* 1991; **81**: 417-420 [PMID: 1721581 DOI: 10.1016/0168-5597(91)90049-4]

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## Update on the treatment of type 2 diabetes mellitus

Juan José Marín-Peñalver, Iciar Martín-Timón, Cristina Sevillano-Collantes, Francisco Javier del Cañizo-Gómez

Juan José Marín-Peñalver, Iciar Martín-Timón, Cristina Sevillano-Collantes, Francisco Javier del Cañizo-Gómez, Section of Endocrinology, Hospital Universitario Infanta Leonor, Facultad de Medicina, Universidad Complutense, 28031 Madrid, Spain

Author contributions: Marín-Peñalver JJ, Martín-Timón I, Sevillano-Collantes C and del Cañizo-Gómez FJ contributed equally to this work.

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Correspondence to: Dr. Francisco Javier del Cañizo-Gómez, Professor of Medicine, Chief of Endocrinology Section, Section of Endocrinology, Hospital Universitario Infanta Leonor, Facultad de Medicina, Universidad Complutense, Avda Gran Vía del Este 80, 28031 Madrid, Spain. [fjcanizog@salud.madrid.org](mailto:fjcanizog@salud.madrid.org)  
 Telephone: +34-91-1918000  
 Fax: +34-91-1918878

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### Abstract

To achieve good metabolic control in diabetes and keep long term, a combination of changes in lifestyle and pharmacological treatment is necessary. Achieving near-

normal glycated hemoglobin significantly, decreases risk of macrovascular and microvascular complications. At present there are different treatments, both oral and injectable, available for the treatment of type 2 diabetes mellitus (T2DM). Treatment algorithms designed to reduce the development or progression of the complications of diabetes emphasizes the need for good glycaemic control. The aim of this review is to perform an update on the benefits and limitations of different drugs, both current and future, for the treatment of T2DM. Initial intervention should focus on lifestyle changes. Moreover, changes in lifestyle have proven to be beneficial, but for many patients is a complication keep long term. Physicians should be familiar with the different types of existing drugs for the treatment of diabetes and select the most effective, safe and better tolerated by patients. Metformin remains the first choice of treatment for most patients. Other alternative or second-line treatment options should be individualized depending on the characteristics of each patient. This article reviews the treatments available for patients with T2DM, with an emphasis on agents introduced within the last decade.

**Key words:** Type 2 diabetes mellitus; Treatment; Oral antidiabetic agents; Injectable antidiabetic agents; Older people; Renal impairment; Future treatments

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**Core tip:** To achieve good metabolic control in diabetes and keep long term, a combination of changes in lifestyle and pharmacological treatment is necessary. Physicians should be familiar with the different types of existing drugs for the treatment of diabetes and select the most effective, safe and better tolerated by patients. This article reviews current and future treatments for patients with type 2 diabetes mellitus, its use in clinical practice and in special situations such as kidney failure and elderly patient, with an emphasis on agents introduced within the last decade.

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## INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a disease that affects more than 400 million people around the world. In 2040, there will be more than 640 million people with diabetes worldwide<sup>[1]</sup>. The prevalence of T2DM is expected to double within the next 20 years, due to the increase of the age, obesity and the number of ethnic groups of high risk in the population<sup>[2]</sup>, with significant increases in cardiovascular disease<sup>[3]</sup>, end-stage renal disease (ESRD)<sup>[4]</sup>, retinopathy and neuropathy. Additionally, to achieve good metabolic control in diabetes and keep long term, a combination of changes in lifestyle and pharmacological treatment is necessary. Achieving near-normal glycated hemoglobin (HbA1c) significantly decreases risk of macrovascular and microvascular complications<sup>[4]</sup>. However, only about 50% of diabetic patients reach their HbA1c target<sup>[5]</sup>. Algorithms for the treatment of diabetes highlight the need for good glycaemic control to reduce the development or progression of diabetes complications. In recent years has increased the number hypoglycaemic agents available for the treatment of T2DM. A recent position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) on a patient-centered approach in the management of patients with T2DM<sup>[6]</sup> gives an overview on how different conditions and co-morbidities may influence the choice of different hypoglycaemic agents. The ADA/EASD suggests that initial intervention should focus on lifestyle changes. Moreover, changes in lifestyle have proven to be beneficial<sup>[7]</sup>, but for many patients is a complication keep long term, due to differing experiences or perceptions<sup>[8]</sup>. In general, drug therapy includes not only initial hypoglycaemic agents, but other intensification strategies to maintain glycaemic control over time, often requiring several drugs with different mechanisms of action<sup>[9]</sup>. Physicians should be familiar with the different types of existing drugs for the treatment of diabetes and select the most effective, safe and better tolerated by patients.

This article reviews current and future treatments for patients with T2DM, its use in clinical practice and in special situations such as kidney failure and elderly patient, with an emphasis on agents introduced within the last decade. The aim of this review is to perform an update on the benefits and limitations of different drugs, both current and future, for the treatment of T2DM.

## LIFESTYLE CHANGES

Dietary intake and physical exercise are the two main

**Table 1** Classification of degree of obesity by body mass index

Body mass index (kg/m <sup>2</sup> )	
Normal weight	18.5-24.9
Overweight grade 1	25-26.9
Overweight grade 2	27-29.9
Obesity grade 1	30-34.9
Obesity grade 2	35-39.9
Obesity grade 3 (morbid)	40-49.9
Obesity grade 4 (extreme)	≥ 50

Adapted from World Health Organization (WHO) 1995, WHO 2000 and WHO 2004.

determinants of the energy balance<sup>[10]</sup>, and they are considered as a basic base in the treatment of patients with diabetes. Adequate rest is also very important for maintaining energy levels and well-being, and all patients should be advised to sleep approximately 7 h per night<sup>[9]</sup>. Evidence supports an association of 6 to 9 h of sleep per night with a reduction in cardiometabolic risk factors<sup>[11]</sup>, whereas sleep deprivation aggravates insulin resistance, hypertension, hyperglycaemia, and dyslipidaemia<sup>[12]</sup>. On the other hand, a screening of patients with suspected obstructive sleep apnoea should be performed, and refer them to a sleep specialist for evaluation and treatment<sup>[9]</sup>.

Although the pharmacological options are each time more extensive and they offer more therapeutics possibilities, especially in the T2DM, the interventions in the life style are essentials in the approach of these patients and they are needed to get the therapeutics goals<sup>[13]</sup>.

### Diet

When nutritional intervention is contemplated, the co-morbidities that can coexist in a diabetic patient also have to be considered. The recommendations on dietary aspects can contribute to achieve the desired blood glucose, blood pressure, lipid profile and weight<sup>[10,14]</sup>, as well as improve sleep apnoea, depression and quality of life related to health; in addition, it has been observed that the incidence of urinary incontinence in women is reduced<sup>[15-18]</sup>.

Numerous randomized controlled trials have demonstrated the metabolic benefits of nutritional recommendations in reducing HbA1c; being variables the results got depending mainly on the length of the disease<sup>[19,20]</sup>.

**Energetic contribution:** Total caloric intake diet will depend on several factors, being determining the presence of overweight or obesity. Body mass index (BMI) is a tool commonly utilized in clinical practice to classify patients and it is calculated by the following equation: [weight (kg)/height (m<sup>2</sup>)] (Table 1).

Most T2DM patients have some degree of overweight or obesity<sup>[21]</sup>. It has been connected to insulin resistance and defects in insulin secretion. These

**Table 2** Different formulas for calculating baseline energy needs of people**Harris-Benedict equation<sup>1</sup>**

Males: BMR (kcal/d) =  $66 + 13.7 \times \text{weight (kg)} + 5 \times \text{height (cm)} - 6.8 \times \text{age}$   
 Females: BMR (kcal/d) =  $655 + 9.6 \times \text{weight (kg)} + 1.8 \times \text{height (cm)} - 4.7 \times \text{age}$   
 Mifflin St Jeor equation<sup>2</sup>  
 Males: BMR (kcal/d) =  $10 \times \text{weight (kg)} + 6.25 \times \text{height (cm)} - 5 \times \text{age} + 5$   
 Females: BMR (kcal/d) =  $10 \times \text{weight (kg)} + 6.25 \times \text{height (cm)} - 5 \times \text{age} - 161$

<sup>1</sup>Harris JA, Benedict FG. Proceedings of the National Academy of Sciences of the United States of America. *Nutr Rev* 1918; **4**: 370-373. <sup>2</sup>Mifflin MD, St Jeor ST, Hill LA, Scott BJ, Daugherty SA, Koh YO. *Am J Clin Nutr* 1990; **51**: 2241-2247. BMR: Basal metabolic rate.

alterations favour the appearance and worsening of diabetes<sup>[22]</sup>, so in these cases in addition to an adequate distribution of macro and micronutrients, we should look for as a main objective a weight reduction by reducing the caloric intake. To achieve this objective, it has been proposed that the caloric intake of the diet prescribed to a diabetic patient with obesity should contain between 500 and 1000 kcal less of its energy needs<sup>[23]</sup>. This weight reduction will improve the insulin sensitivity, being a favourable factor to improve the glycaemic control parameters<sup>[24]</sup>. In the case of patients for whom there is no excess weight, the diet should be isocaloric.

There are different formulas for calculating baseline energy needs of people (Table 2). To these basal needs, a factor depending on the physical activity must be added. The randomized trial LOOK AHEAD, showed that weight loss after an intervention in lifestyles, improve blood pressure, and blood glucose control and lipid profile<sup>[25]</sup>, especially in patients with a recent diagnosis of disease<sup>[3]</sup>. When this study was prolonged, it was found that intensive nutritional intervention did not provide an improvement in the rate of cardiovascular events or weight loss when it is compared against a standard nutritional intervention<sup>[26]</sup>.

**Macronutrient distribution:** There is not enough evidence to suggest an ideal percentage in the distribution of carbohydrates, lipids and proteins. There are several studies that have sought to distribute the best ratio macronutrients without finding valid results, and several dietary patterns that have been analysed as the Mediterranean diet, vegetarian or vegan diet, Dietary Approaches to Stop Hypertension (DASH), low-fat diet and low carbohydrates diet observing a modest effectiveness of managing diabetes. The benefits happen only when they are accompanied by a lose weight so more studies are needed<sup>[27]</sup>.

**Carbohydrates:** Although there is no consensus on the percentage of carbohydrates that people with diabetes should eat, it has been shown that the amount and the type of carbohydrates are the main determinants for glycaemic control. Counting carbohydrates has proven to be very important in all patients. It allows

**Table 3** Glycaemic index, glycaemic load and carbohydrates portion

GI: Observed increase in blood glucose after eating 50 g of a food, compared with the observed increase after intake of 50 g of white bread or glucose  
 Glycaemic load: GI  $\times$  total amount of carbohydrates (grams) of the usual food portion  
 Carbohydrates portion: amount of food containing 10 g of carbohydrates

Glycaemic Research Institute. Available from: URL: <http://www.glycemic.com/GlycemicIndex-LoadDefined.htm>. GI: Glycaemic index.

a better adjustment of the postprandial blood glucose for those who take insulin. With this method, patients consumed a known amount of carbohydrates divided among different meals and calculated it in grams of carbohydrates per portion (Table 3). This type of measurement is more important in patients with basal-bolus treatment or with continuous insulin infusion<sup>[28]</sup>.

It is preferable that the intake of carbohydrates comes from products such as fruits, vegetables, legumes, whole grains and dairy vs those involve the added contribution of salt, fat or simple sugars<sup>[10]</sup>.

**Index and glycaemic load:** There is large confusion in the interpretation about the effect of the diet with low glycaemic index and there is not unanimity in the results of the different studies. Even though these diets are recommended by some associations because there are studies in which have been observed a better glycaemic control when it is compared above all with high glycaemic index food<sup>[29]</sup>, there are articles that have questioned this assertion. They based this divergence on: The different definition of glycaemic index, they do not take into account the fiber contribution, and the different glycaemic response to the same food in different individuals. They consider that cannot be determinate that the observed effect is exclusively due to the food's glycaemic load<sup>[30]</sup> (Table 3).

**Fiber:** Dietary fiber intake, especially the fiber that provide the natural resources, has shown that improve the control of cardiovascular risk factors, and improved the glycaemic control, turning into a lower risk of cardiovascular mortality in people with diabetes<sup>[27,31]</sup>. However, some studies have shown that the effect on diabetes has a modest significance and it is achieved with high amounts of fiber a day but this is far away from a real consumption in daily life (greater than 50 g/d)<sup>[32]</sup>.

Generally, and taking into account the modest beneficial effects on cardiovascular risk factors, in diabetic patients is suggested a consumption of fiber and whole grains at least similar to that recommended for the general population; about 25 g/d for women, and 38 g/d for men or 14 g per 1000 kcal<sup>[28]</sup>.

**Sucrose and fructose:** Contrary to what one might

think sucrose intakes of 10%-35% of total energy do not have a negative effect on glycaemic or lipid responses when sucrose is substituted for isocaloric amounts of starch<sup>[33]</sup>. Consume free fructose (naturally occurring from foods such as fruit) did not get worsen the glycaemic control more than other forms of sugar, although it should avoid further intake of 12% of daily calories<sup>[28]</sup>. Restriction is advised of these sugars in the diet to avoid excessive caloric intake that can contribute to weight gain if are taken in large quantities. Moreover, sugary drinks contain large amounts of fast absorbing carbohydrates and have demonstrated a cardiovascular risk and diabetes increase in the healthy population that consumes them. Especially harmful when are sweetened with fructose free. Although there are not many studies in diabetic patients, there is no reason to think they will not have the same consequences. Therefore, the consumption of these drinks is contraindicated<sup>[34]</sup>.

**Non caloric sweeteners:** Opposite of natural simple sugars there are sweeteners with lower calorific value. Most are artificial. They do not have caloric contribution, except aspartame (containing 4 kcal/g), and do not increase blood glucose. These sweeteners can be used by diabetic patients. If they are employed to replace glucose, bring the benefit of reducing the kilocalories in the diet<sup>[35]</sup>.

**Proteins:** It is interesting to make a differentiation between diabetic patients with and without kidney disease. In people without kidney disease, protein intake usually recommended is between 15%-20%; however, reviewing scientific studies no firm conclusion could be reached with respect to this issue. In the literature we can find different randomized clinical trials faced on this issue results. On the one hand there are studies that demonstrate that if 28%-40% of the energy of the diet is taken as proteins there is an improvement of the HbA1c, triglycerides, total cholesterol and/or LDL cholesterol<sup>[36]</sup>, while others studies have not shown a benefit in any of these aspects<sup>[37]</sup>. In patients with kidney disease, whether if we refer to micro or macroalbuminuria, reducing protein intake below the usual has been undergone various tests and meta-analysis and the evidence has not shown that improve glycaemic control, cardiovascular risk factors or renal disease progression following low-protein diets<sup>[27]</sup>. With regard to the origin of proteins, there is no difference between animal and vegetable origin in relation to proteinuria<sup>[28]</sup>.

Finally, the proteins in patients with T2DM, although they do not have effect on blood glucose control itself, seems to increase the insulin response so it is not advisable to use proteins in situations of hypoglycaemia.

**Fat:** Epidemiological studies have related fats with the risk of developing obesity and cardiovascular risk<sup>[38]</sup>. As in the rest of immediate principles there is

no optimal fat proportion and, as a general rule, the recommendations for the general population (between 20%-35%) are applied for diabetic patient, paying special attention if the patient is overweight, then the percentage should be at the lower limits. Despite these recommendations, diabetic patients often take more fat than the recommended<sup>[39]</sup>.

We can distinguish between saturated and unsaturated fats (monounsaturated and polyunsaturated). In addition, has to be specified that trans fatty acids may be a type of unsaturated fat but with harmful effects on the body for its different structure. Distinguish between these types is important because it has been demonstrated that the quality is more relevant than the amount of fat consumed.

There are few studies in diabetic patients about consumption of saturated fatty acids or cholesterol; in this regard the recommendations for patients with diabetes are the same as for the general population: A contribution of saturated fat < 10%, with a minimum intake of trans fatty acids and with a contribution of cholesterol < 300 mg/dL<sup>[10]</sup> preferably choosing monounsaturated and polyunsaturated fatty acids (including omega-3 fatty acids). Some studies, that have studied the Mediterranean dietary pattern, have demonstrated that monounsaturated fatty acids can improve cardiovascular risk factors and glycaemic control<sup>[40]</sup>, especially if they are replaced with saturated fatty acids.

**Omega-3 fatty acids:** Although there are unlike results, in general we cannot say that omega-3 supplements have shown clear cardiovascular benefit<sup>[41]</sup>. However, consumption of products high in omega-3 can be positive in preventing cardiovascular disease<sup>[42]</sup>.

**Alcohol:** Alcohol should be drunk in moderation and it should not exceed one serving per day for women, or two servings per day in the case of men. To avoid excess of energy when they are consumed, this contribution must be exchanged for other products. This moderate consumption does not harm the glycaemic control but rather in some studies has been found the contrary, with moderation can improve glycaemic control and reduce cardiovascular events.

Despite the above facts, it is very important to note that alcoholic beverages may contribute to the appearance of late hypoglycaemia especially in patients in treatment with hypoglycaemic drugs, so we should warn the patient to pay attention to any symptoms of hypoglycaemia<sup>[28]</sup>.

**Sodium:** The recommendation for the general population to reduce sodium intake to less than 2300 mg/d shall also apply to patients with diabetes mellitus. When these also have hypertension, which is very common, reduced sodium intake should be individualized<sup>[43]</sup>.

**Specific supplements:** The potential benefits of



**Table 4 Relationship between maximum oxygen consumption, % of maximum heart rate and subjective perceived exertion**

Intensity	% oxygen consumption	% maximum heart rate <sup>1</sup>	Subjective perceived exertion
Very light	< 20	< 35	< 10
Light	20-39	35-54	10-11
Moderate	40-59	55-69	12-13
High	60-84	70-89	14-16
Very high	> 85	> 90	17-19
Maximum	100	100	20

<sup>1</sup>Maximum heart rate = 220-age. Available from: URL: American Diabetes Association. Physical Activity/Exercise and Diabetes. *Diabetes Care* 2004; 27 (Suppl 1): S58-S62.

dietary supplements for diabetic patients with various specific nutrients have been subjected to trials. In spite of this, reliable data has not been observed to confirm benefits in glycaemic control supplementing because of supplement the diet with antioxidants as vitamin and carotenes, micronutrients such as chromium or other herbs. The recommendations of vitamins and minerals are not different from the general population, they are provided by a varied diet<sup>[38]</sup>.

### Exercise

The physical activity and exercise are one of the basic strategies in the treatment of diabetes. Promoting exercise, within a specific plan, provides in general terms multiple benefits: Increased insulin sensitivity in tissues, improvement of glycaemic control<sup>[44]</sup>, benefits in lipid profile and blood pressure, maintenance or weight loss, cardiovascular benefits, better quality of life, psychological well-being and improvement of depression<sup>[10]</sup>.

**Benefits of glycaemic control:** In some studies it has observed a significant decrease in HbA1c in patients with T2DM who do exercise. The difference in the degree of improvement observed in the different studies will depend on the characteristics of the patient and the type of training, thus, it is more effective when training programs are based on aerobic exercises of programs based on muscle strength in isolation<sup>[45]</sup>.

**Other benefits:** The physical exercise also brings improvement in other metabolic parameters. It helps control cardiovascular risk factors (dyslipidaemia, hypertension, weight maintenance, psychological benefits, reduces mortality, improvement cardiorespiratory fitness and peripheral neuropathy<sup>[10,45]</sup>).

**Types of exercise:** Both aerobic and resistance exercises have demonstrated benefits in people with diabetes through increased glucose uptake and decreased insulin resistance.

Though aerobic exercise in isolation seems to get better benefits than resistance exercise<sup>[45]</sup>, in patients with diabetes is recommended the combination of both

types because the effect is greater than if each one is performed in isolation<sup>[46,47]</sup>.

This type of training has been traditionally recommended for patients with T2DM. A frequency of at least 3 d per week is recommended, preferably if it can be increased to 5 d with no more than two consecutive days between periods of activity, because the increase of the sensitivity and the glucose tolerance is maintained for about 12-24 h. It should be done with moderate intensity which is 40%-60% of maximum aerobic capacity. This corresponds to 55%-69% of maximum heart rate according to age (maximum heart rate = 220-age)<sup>[47]</sup>. Another method for measuring the intensity can be the subjective perception of the effort that assigns values to 20 points according to the patient judgment about the activity performed (Table 4). A moderate-intensity exercise can also be an activity that can be conducted while maintaining an uninterrupted conversation.

The effect of exercise in T2DM is clearly related to the volume done, thus, in different societies, it is recommended at least a minimum of 150 min per week<sup>[43,47]</sup>. Despite following the same recommendations, it has recently published a review where it is expounded that shorter performance exercises, with reference to the accumulated time during the week, keeps some benefit although this is less<sup>[48]</sup>.

This type of exercise should be performed 2-3 times a week on non-consecutive days. For optimal gains in strength and insulin action, training should be moderate (50% of 1 repetition maximum) or vigorous (75%-80% of 1 repetition maximum). Each session should include from 5 to 10 exercises involving the use of large muscle groups. Ten to fifteen repetitions of each exercise (30-45 s) have to be made. Between each series should be left between 1-2 min for the recovery. Supervision by a professional can ensure an appropriate enforcement and progression of the exercise that optimized the benefits and reduce the risk of complications<sup>[47]</sup>.

Although they have not demonstrated benefits in glycaemic control, these exercises are also recommended and can be very useful in older patients with T2DM<sup>[49]</sup>.

**Unstructured physical activity:** It is also recommended to advise patients to increase energy expenditure in activities of daily life. It requires an increase of unstructured physical activity (walking more in the day, climb the stairs...)<sup>[50]</sup>.

**Prescription of a specific plan:** Exercise should be prescribed individually for each patient and taking into account the characteristics of the person. Initially, the guidelines should recommend a slow progression and, if it is necessary, the patient has to start with low volumes of work. Recommendations should take into account the type of diabetes and the treatment utilized, the possibility that patients have diabetic foot, retinopathy, neuropathy, nephropathy or some

degree of cardiovascular risk<sup>[49]</sup>. Training plans that are supervised by professionals have proved to be more effective as this study have demonstrated. In it, is compared a supervised program against a general advice, and although in both an increase in physical activity is observed, some better effects in HbA1c and cardiovascular risk factors in the supervised group have been seen<sup>[51]</sup>.

Before starting the exercise would be advisable to pre-clinical evaluation, paying special attention to physical ability, complications of diabetes and comorbidities that constrain the realization of physical activity. For patients at high cardiovascular risk or for those who start high-intensity exercises, the ADA recommends performing an effort test with a grade of recommendation C<sup>[47]</sup>.

**Exercise and diabetes complications:** The presence of diabetes complications involves a number of considerations at the time of writing prescriptions of physical exercise in these patients.

The physical exercise has proved benefits in reducing the appearance of peripheral neuropathy<sup>[52]</sup>. When it is already present, it is recommended to avoid exercises that cause impacts of repetition in the lower extremities and especially in patients with foot ulcers and wounds<sup>[53]</sup>. Furthermore, recent studies have demonstrated that moderate intensity walking do not increase the risk of ulcers.

In respect of the weight-bearing exercises, it can be performed while there are no ulcers or foot lesions. In any case it should pay attention and examine the feet and always wear suitable shoes.

The presence of retinopathy advises against the practice of physical activities that increase intrathoracic pressure (Valsalva manoeuvre), or high-intensity exercises by the risk of retinal detachment or intravitreal haemorrhage. The exercises with low and moderate intensity (walking, swimming...) are perfectly authorized and they can be done safely. Contact exercises like boxing should be avoided because of the risk of impact<sup>[50]</sup>.

Exercise for diabetic patients is beneficial at any stage of renal function. In epidemiological studies it has been shown to improve renal function. Promotes muscle strengthening in case of kidney failure that helps to counteract sarcopenia, and improves various parameters in patients on dialysis, so with supervision and restraint exercise is recommended and although they have been transient increases in microalbuminuria with sessions of exercise (because of increasing blood pressure) is not considered as a marker of persistent microalbuminuria<sup>[50]</sup>.

Physical activity has many beneficial cardiovascular effects but must take into account some considerations when there is vascular disease. Patients with diabetes that present a moderate or high cardiovascular risk should be included in supervised cardiac rehabilitation programs, because exist an association with mortality.

In addition, during the exercise there is an increased activity of the sympathetic nervous system and catecholamines and decrease vagal tone<sup>[47,50]</sup>.

In people with peripheral arterial disease benefits from the practice of sports aerobics and resistance also exist because of the improvement of the mobility, functional capacity, pain tolerance and quality of life<sup>[47]</sup>.

Moderate physical exercise can improve the autonomic nervous system both in patients with autonomic neuropathy and those who do not have it<sup>[54]</sup>, however it may represent a prescription limitation because it may favour silent ischemia, doubling mortality, impairing exercise tolerance and decreasing the maximum heart rate and thus a prior cardiovascular study is recommended<sup>[55]</sup>.

### **Exercise with uncontrolled blood sugar**

**Hyperglycaemia:** In T2DM is very strange developing a true insulin deficiency, as in type 1 diabetic, so if the patient feels well is not necessary to postpone the exercise by hyperglycaemia, although they must ensure an adequate hydration state<sup>[56]</sup>.

In non-diabetic person with aerobic exercise the increase of the glucose uptake is offset with similar increase of the hepatic glucose, but in diabetic person the muscle uptake is greater than the liver's production although the risk of hypoglycaemia is minimal if hypoglycaemic drugs are not taken<sup>[47]</sup>. However, if in addition to the effect of exercise add up the effects of hypoglycaemic drugs, we recommend a series of precautions mainly based on carbohydrate intake and adjust drug doses. If the levels before exercise are less than 100 mg/dL should take a supplement of 15 g of carbohydrates before exercise. This measure should only be recommended if blood glucose lowering drugs (secretagogues or insulin) are taken. If the control is with other drugs, supplements are not required if the exercise is less than an hour<sup>[56]</sup>. It is important to note that regardless of the initial levels, if the exercise is prolonged a monitoring could be required and also intakes over the same period.

Before physical activity, to prevent the appearance of hypoglycaemia during exercise, doses of drugs such as insulin secretagogues or insulin (especially the latter) can be decreased. These measures can be associated with dietary measures mentioned above. During the hours after exercise glucose needs increase, so after exercise delayed hypoglycaemia can happens. This hypoglycaemia should be expected and may require reducing the dose of drugs after exercise and/or increase the intake after it<sup>[47]</sup>.

## **ORAL AGENTS**

### **Metformin**

Metformin is considered the agent of first line for treatment of T2DM, in the absence of contraindications<sup>[6,13,57]</sup>.

**Mechanism of action**<sup>[58]</sup>: Metformin can change

the composition of gut microbiota<sup>[59]</sup> and activate mucosal AMP-activated protein-kinase (AMPK) that maintain the integrity of the intestinal barrier. These effects, in combination with the activation of AMPK<sup>[60]</sup> in hepatocytes appear to be the mechanism by which metformin decrease lipopolysaccharide (LPS) levels in circulation and in the liver.

After being delivered to the liver from the intestines, metformin can inhibit gluconeogenesis through four different mechanisms<sup>[61]</sup>: (1) by activating hepatic AMPK through liver-kinase B1 and decreased energy charge (9, 10); (2) through the inhibition of glucagon-induced cAMP production by blocking adenylcyclase (11); (3) in high concentrations (5 mmol/L) inhibit NADH coenzyme Q oxidoreductase (complex I) in the mitochondrial electron transport chain (12) to reduce ATP levels and increase AMP/ATP ratio. This increased ratio should activate AMPK; and (4) the inhibition of mitochondrial glycerol phosphate dehydrogenase (mG3PDH)<sup>[58]</sup>, will affect transport of NADH from the cytoplasm into mitochondrion, suppressing gluconeogenesis process from lactate.

Also, metformin works through the Peutz-Jeghers protein LKB1. LKB1 is a tumour suppressor, and activation of AMPK through LKB1<sup>[62]</sup> may play a role in inhibiting cell growth.

**Indications and contraindications:** Metformin is the drug of first-line for many patients with T2DM. It decreases fasting blood glucose by approximately 20% and HbA1c by 1.5%. It can be given in combination with sulfonylureas, glinides, alpha-glucosidase inhibitors, insulin, thiazolidinediones (TZD), glucagon-like peptide-1 receptor agonist (RA-GLP1), dipeptidylpeptidase 4 inhibitors (iDPP4), and sodium-glucose co-transporter 2 inhibitors (iSGLT2). Metformin is contraindicated in patients with factors that predispose to lactic acidosis. The predisposing factors are: A renal function damaged, concomitant liver disease or excessive alcohol intake, unstable or acute heart failure and personal history of lactic acidosis.

The precise serum creatinine and estimated glomerular filtration rate (eGFR) limits for the use of metformin remain uncertain. In the metformin prescribing information is contraindicated when creatinine level is above 1.4 mg/dL in woman and 1.5 mg/dL in men, and with eGFR < 60 mL/min. However, in observational studies of T2DM patients and eGFR 45-60 mL/min, improved clinical outcomes have been reported. Nowadays<sup>[63-65]</sup>, in patients with eGFR above 45 mL/min, metformin can be utilized. The absolute contraindication is with GFR < 30 mL/min. With eGFR 30-45 mL/min, in clinical practice, currently we reduce metformin dose by a half. It is very important to advise patients with eGFR 30-60 mL/min to stop taking metformin if they develop any condition associated with dehydration, sepsis or hypoxemia. Also metformin should be stopped prior to intravenous iodinated contrast.

**Side effects:** The most frequent are gastrointestinal, such as anorexia, nausea, abdominal discomfort and diarrhoea; they are usually mild and transient. Also, metformin reduces intestinal absorption of vitamin B12.

Less common is lactic acidosis. In a review<sup>[66]</sup> of 347 randomized trials and prospective cohort studies, there were no cases of lactic acidosis. However, is very important because of the high case-fatality rate. Predisposing factors are all situations that predispose to hypoperfusion and hypoxemia (sepsis, heart failure, dehydration, acute or progressive renal impairment).

**Cardiovascular effects:** Metformin does not have adverse cardiovascular effects, and it appears to decrease cardiovascular events as we saw in UKPDS, and during the post-interventional observation period of the UKPDS, in which reductions in the risk of macrovascular complications were maintained in the metformin group.

Metformin also has a lipid-lowering activity, and it result in a decrease in free fatty acid concentration, serum triglyceride, small decrease in LDL cholesterol and a modest increase in HDL cholesterol.

**Cancer incidence:** Observational data suggest that metformin decreases cancer incidence<sup>[67,68]</sup>. In different meta-analyses in T2DM patients, use of metformin compared with non-use or with use of other diabetes treatment, was related with a reduced risk of all cancers and lower cancer mortality<sup>[69,70]</sup>. The majority of the trials were not designed to explore cancer outcomes, so we must be prudent in the interpretation of their results.

#### **Insulin secretagogues: Sulfonylureas and meglitinides**

Sulfonylureas and meglitinides or glinides (insulin secretagogues) are two different classes of oral hypoglycaemic drugs but they have a common mechanism of action, and both stimulate pancreatic beta cells to release insulin.

Sulfonylureas are a classic first or second-line therapy for patients with T2DM<sup>[71]</sup>, and since their introduction to clinical practice in the 1950s they have been widely utilized<sup>[72]</sup>. They are utilized as a reference to compare the efficacy and safety of other hypoglycaemic drugs excluding insulin.

Meglitinides stimulate insulin release through similar mechanisms but they have a different subunit binding site, with a more rapid absorption and more rapid stimulus to insulin secretion. However they require more frequent dosing<sup>[73]</sup>.

**Mechanism of action:** Both sulfonylureas and glinides base their mechanism of action in increasing insulin secretion, which is regulated by ATP-sensitive potassium channels (KATP potassium channel) located in the membrane of pancreatic beta cells<sup>[74]</sup>. Although the receptor's binding site is different for sulfonylureas

and glinides, they both induce channel closure and cell depolarization leading to an increase in cytoplasmic calcium level and consequently insulin secretion<sup>[37]</sup>.

**Pharmacokinetics:** Differences in pharmacokinetic and binding properties of insulin secretagogues result in the specific responses that each drug produces. Sulfonylureas can be divided into first- and second-generation agents. Glyburide (known as glibenclamide in Europe), glipizide, gliclazide and glimepiride are second-generation sulfonylureas<sup>[57]</sup>. New generation agents are more potent and have fewer adverse effects<sup>[37]</sup>. Although second-generation sulfonylureas are equally effective, there are differences in absorption, metabolism, and duration of action as well as in effective dose; for example, glyburide has active metabolites that can prolong his action.

There are two different glinides: Repaglinide and nateglinide. Repaglinide is a member of the meglitinide family different from the sulfonylurea. Nateglinide is a derivate of phenylalanine and it is structurally difference from sulfonylureas and meglitinide. They both cause less hypoglycaemia and less weight gain due to their shorter half-life and a different sulfonylureas receptor binding site, leading to faster absorption and a more rapid stimulus to insulin secretion<sup>[37]</sup>.

As a result of their pharmacokinetics, the major effect of sulfonylureas is the reduction of fasting plasma glucose concentrations, whereas meglitinides mainly reduce postprandial glucose<sup>[75]</sup>.

**Advantages and effectiveness:** Sulfonylureas and meglitinides can be effective when employed as monotherapy, or in combination with other oral hypoglycaemic drugs or insulin. Sulfonylureas are the most cost-effective glucose-lowering agents, have been on the market for a long time<sup>[37]</sup>, and are widely utilized because of their long term efficacy and safety history, low cost and extensive clinical trial data demonstrating good glucose-lowering efficacy<sup>[76,77]</sup>. The glucose-lowering effectiveness is said to be high for sulfonylureas (expected HbA1c reduction 1.0%-1.5%) and generally lower for meglitinides (0.5%-1.0%)<sup>[9,57]</sup>.

In the Consensus of ADA/EASD 2015 sulfonylureas and glinides appear as an alternative to metformin when metformin is contraindicated or not tolerated, and they represent an alternate treatment option in double and triple therapy<sup>[57]</sup>, whereas in the Consensus of the American Association of Clinical Endocrinologist (AACE) 2016, sulfonylureas and glinides appear as the last alternative both in monotherapy and combined treatment<sup>[9]</sup>.

**Side effects:** Loss of efficacy, hypoglycaemia and weight gain represent the main problems related to the use of these drugs.

Over time insulin secretagogues lose effectiveness (secondary failure), caused by an exacerbation of islet dysfunction with beta cell failure<sup>[78,79]</sup>. As a result, the

percentage of patients maintaining adequate glycaemic control decreases progressively. Although this effect may also be related to disease progression, it has shown an increase in secondary failure than other agents<sup>[80]</sup>.

Weight gain can be *via* many of the same mechanisms that are triggered by insulin therapy, and it has been observed in different studies<sup>[81,82]</sup>. However, metformin might counter the weight gain effect when used in combination<sup>[81,83]</sup>. Different generations of sulfonylureas have shown to cause weight gain and its magnitude appears to correlate with the propensity to cause hypoglycaemia. It may also occur with meglitinides as they have similar profiles<sup>[76]</sup>, but it seems to occur in a lesser extent due to their short action<sup>[78]</sup>.

Hypoglycaemia is the most common adverse effect<sup>[83,84]</sup>, especially with long-acting sulfonylureas (such as glyburide/glimepiride)<sup>[85]</sup>. New generation sulfonylureas have shown to have a significantly lower risk of hypoglycaemia. Meglitinides generally have less risk of hypoglycaemia<sup>[37]</sup>, thus being useful for individuals in whom the goal of avoiding hypoglycaemic events is important.

The risk factors for hypoglycaemia are inconsistent eating patterns in older individuals (meglitinides can be useful in these patients), malnutrition, alcohol ingestion, renal insufficiency, hepatic failure, hypothyroidism or drug interactions<sup>[86,87]</sup>. The risk of hypoglycaemia, as well as considerations of the risk-to benefit-relationship, is particularly relevant in older individuals where results from trials have suggested that aggressive control may not have significant benefits and may present some risk<sup>[6]</sup>.

**Cardiovascular disease:** Sulfonylureas have been associated with increased cardiovascular risk, especially when it comes to glyburide/glibenclamide. Some studies<sup>[88,89]</sup> support this association, which can be explained by the interference with ischemic preconditioning, a protective autoregulatory mechanism in the heart. However, other studies like UKPDS, ADVANCE and ACCORD and many meta-analyses failed to proof an increased risk in cardiovascular mortality or morbidity<sup>[76]</sup>. Therefore, it remains unclear whether sulfonylureas are associated with an increased cardiovascular risk but as glibenclamide may indeed be when compared with other sulfonylureas, clinicians should consider possible differences in risk of mortality if a sulfonylurea is to be utilized.

**Other considerations:** Most insulin secretagogues undergo significant renal clearance except for meglitinides, and the risk of hypoglycaemia is higher in patients who have chronic kidney disease (CKD) especially with glyburide/glibenclamide which has a prolonged duration of action and active metabolites<sup>[58]</sup>. In patients with liver disease, sulfonylurea is not specifically contraindicated and meglitinides can also be employed. When liver disease is severe, insulin



secretagogues have an increased risk of hypoglycaemia and should be avoided<sup>[57,90]</sup>.

Sulfonylureas have several drug-drug interactions as they are metabolized by cytochrome p450<sup>[84]</sup>. Repaglinide with gemfibrozil is contraindicated because of its higher risk of hypoglycaemia.

### Alpha-glucosidase inhibitors

There are three currently available agents, acarbose, miglitol and voglibose<sup>[37]</sup>. Their properties are different from other antidiabetics owing to its unique mode of action. Acarbose has been used for over 20 years in the treatment of hyperglycaemia<sup>[91]</sup>.

The alpha-glucosidase inhibitors reduce postprandial triglycerides but their effect on LDL and HDL cholesterol levels and fasting triglycerides is insignificant and inconsistent<sup>[75,92]</sup>. Alpha-glucosidase inhibitors rarely induce hypoglycaemia, because these agents do not stimulate insulin release, and do not significantly affect body weight<sup>[82]</sup>.

Acarbose has demonstrated to have beneficial effects by reducing the risk of cardiovascular disease and slowing the progression to diabetes in patients with impaired glucose tolerance<sup>[93,94]</sup>.

**Mechanism of action:** Alpha-glucosidases are enzyme complexes located in the brush border membrane of the small intestine and hydrolyse oligosaccharides into monosaccharides<sup>[95]</sup>. Alpha-glucosidases inhibitors are structurally similar to natural oligosaccharides with higher affinity for alpha-glucosidases<sup>[91]</sup>, and they produce a reversible inhibition of membrane-bound intestinal alpha-glucoside hydrolase enzymes. This cause delayed carbohydrate absorption and digestion, and results in a reduction in postprandial hyperglycaemia. The undigested carbohydrates in the lower parts of the small intestine increase plasma RA-GLP1 levels<sup>[95]</sup>. Because reduced blood glucose concentrations, alpha-glucosidase inhibitors do not enhance insulin secretion<sup>[91,95]</sup>.

**Efficacy:** In general, alpha-glucosidase inhibitors have modest HbA1c lowering effects. In the Consensus of ADA/EASD 2015, alpha-glucosidase inhibitors are not included in the algorithm due to their lower efficacy and limiting side effects compared to other options<sup>[57]</sup>, whereas in the Consensus of AACE 2016, alpha-glucosidase inhibitors appear only before sulfonylureas and glinides as monotherapy and combined treatment<sup>[9]</sup>.

**Side effects:** The side effects are mainly gastrointestinal and include flatulence, diarrhoea and abdominal pain. These symptoms are usually mild, but they may reduce compliance and they are the most common reason for discontinuation treatment<sup>[94,95]</sup>. These symptoms occur when undigested carbohydrates arrive to the colon and as a result, there is a fermentation by bacteria in the large bowel and intestinal gas production<sup>[91]</sup>. For this reason, they are contraindicated in patients with chronic intestinal disorders associated with impaired digestion or

absorption, and with conditions that may worsen when an intestinal gas increase appears (hernias, intestinal obstruction and intestinal ulcers). Treatment should be discontinued immediately if there is or is suspected ileus or sub ileus. To maximize the potential for these agents to be well tolerated, start with a low dose and increase slowly<sup>[37]</sup>.

Alpha-glucosidase inhibitors are not recommended for patients with creatinine clearance < 25 mL/min and they can produce asymptomatic elevation of liver enzymes, for this it is necessary a control of liver enzymes<sup>[96]</sup>. In hypoglycaemia (when it is associated with sulfonylureas, glinides and insulin), like inhibitors of  $\alpha$ -glucosidase delay absorption and digestion of sucrose, patients must take glucose.

### Thiazolidinediones

Two TZD are currently available in United States: Rosiglitazone and pioglitazone. In Europe, since 2010, rosiglitazone was suspended by the European Medicines Agency, based on the overall risks of rosiglitazone exceed their benefits. French and Germany Medicines Agencies also discontinued pioglitazone in 2011.

**Mechanism of action:** TZD increase insulin sensitivity by acting on muscle, adipose tissue and liver to increase glucose utilization and decrease glucose production. TZD bind to peroxisome proliferator-activated receptors (PPARs). PPAR- $\gamma$  is found predominantly in central nervous system, macrophages, vascular endothelium, adipose tissue and pancreatic beta-cells. The concentration of PPAR gamma is increased in the skeletal muscle of obese and diabetic patients<sup>[97]</sup>. In the central nervous system PPAR-gamma activation mediates weight gain by stimulating increased feeding<sup>[98]</sup>; this is, in part, the reason for weight gain associated with TZD.

PPAR-alpha is found predominantly in liver, skeletal muscle, heart and vascular walls. Rosiglitazone is purely PPAR-gamma agonist, while pioglitazone has also some PPAR-alpha effects; therefore they have different effects on lipids. Pioglitazone produces a more favourable lipid profile: LDL-cholesterol remained constant during treatment while rosiglitazone raises them; in addition decreased more triglyceride levels than rosiglitazone. HDL-cholesterol increased more or less 10% with both of them.

TZD also may improve blood glucose levels by preserving pancreatic beta-cell function. They are probably similar in efficacy to metformin in monotherapy but we don't usually choose them because of their adverse effects and cost. Also, they are effective in combination therapy, but again, we typically prefer combination with other drugs with less adverse effects. TZD should not be given to diabetic patients with a history of heart failure or low bone mass.

The ratio between benefit and risk at cardiovascular system of rosiglitazone and pioglitazone remains unclear. Meta-analyses and observational studies (RECORD study, BARI 2D, PROactive trial) suggest caution with

rosiglitazone use and also with pioglitazone.

### Side effects

**Weight gain:** The weight gain is the result of diverse mechanisms as: Fluid retention, the activation of PPAR- $\gamma$  in the central nervous system (which increases feeding) and the up regulation of genes that facilitate adipocyte lipid storage (in part weight gain may be also a result from the proliferation of new adipocytes<sup>[99]</sup>). It's time and dose dependent.

**Heart failure:** PPAR- $\gamma$  is more abundant in the collecting tubules of the nephron; the PPAR-gamma stimulation (induced by TZD treatment) activate sodium reabsorption in the luminal membrane of the collecting tubule cells<sup>[100]</sup>, leading to a fluid retention that may lead to the precipitation of heart failure or worsening it. Peripheral oedema occurs in 4%-6% of patients in treatment with TZD, and this percentage is higher in patients with heart failure history. Because of the risk of heart failure the American Heart Association and the ADA published a consensus statement in 2003<sup>[101]</sup>.

Because of their mechanism of action (they improve blood glucose by increasing insulin sensitivity) TZD monotherapy cause hypoglycaemia less frequently than sulfonylureas or insulin.

In preclinical studies pioglitazone increased bladder tumours in rats. Latter the Prospective Pioglitazone Clinical Trial in Macrovascular Events (PROactive) saws more cases of bladder cancer: 14 vs 5, in the treatment group<sup>[102]</sup>. In an analysis of an ongoing 10-years observational study, there wasn't a significant association between pioglitazone and cancer<sup>[103]</sup>, but the risk of bladder cancer was significantly increased in those with the longest exposure and highest cumulative dose. Using data from the Adverse Event Reporting System of the United States FDA, again risk of bladder cancer was higher with pioglitazone<sup>[104]</sup>. Because of these in 2011 German and French Medicines Agencies suspended the use of pioglitazone.

Decrease bone density and increase fracture risk. The activation of PPAR-gamma has been demonstrated to down regulate components of the IGF-1 system, and IGF-1 is an important regulator of osteoblast proliferation and differentiation<sup>[105]</sup>. The absolute increase in risk fracture seems to be small and occurred with both of them, rosiglitazone and pioglitazone; the fractures are more frequently in the distal upper or lower extremities. These treatments should not be utilized in women with low bone density or with risk factors for fracture.

Troglitazone suspended its commercialization due of severe hepatocellular injury<sup>[106]</sup>. FDA currently recommends periodic monitoring of liver function in patients in treatment with rosiglitazone or pioglitazone.

### Dipeptidyl peptidase-4 inhibitors

The incretin agents (GLP1 and GIP), secreted by intestine L cells, increase insulin secretion and inhibit

glucagon in response to nutrient inputs. The glucoregulatory effects of incretins are the basis for treatment with inhibitors of DPP4 in patients with T2DM. Agents that inhibit DPP4, an enzyme that rapidly inactivates incretins, increase active levels of these hormones and, in doing so, improve islet function and glycaemic control in T2DM.

iDPP4 are used as monotherapy in patients inadequately controlled by diet and exercise, and dual therapy in combination with metformin, TZDs and insulin. iDPP4 are well tolerated; they have a low risk of producing hypoglycaemia, and maintain the patient's weight. We have five iDPP: Sitagliptin, Vildagliptin, Saxagliptin, Linagliptin and Alogliptin.

**Sitagliptin:** Sitagliptin, which is approved for the treatment of T2DM in many countries, can be employed alone or dual therapy with sulfonylurea, metformin or TZD or third therapy. The normal dose of sitagliptin is 100 mg once daily; half dose is utilized in patients with an eGFR 30-50 mL/min, and quarter dose in those with an eGFR < 30 mL/min<sup>[107]</sup>.

Monotherapy with this drug there are multiple studies, with significant reduction in HbA1c. The results of a study with sitagliptin monotherapy for 18 wk were: HbA1c significantly decreased with sitagliptin 100 and 200 mg compared to placebo (low HbA1c vs placebo: -0.48% and -0.60% respectively). Sitagliptin also significantly reduced fasting blood glucose vs placebo. Patients with baseline HbA1c higher (> or = 9%) had greater reductions in HbA1c subtracted sitagliptin placebo (-1.20% for 100 mg and -1.04% in the case of 200 mg) than those with HbA1c < 8% (-0.44% and -0.33%, respectively) or > or = 8% to 8.9% (-0.61% and -0.39%, respectively). Sitagliptin had a neutral effect on body weight<sup>[108]</sup>.

In dual therapy studies the results confirm that sitagliptin was as effective as glipizide in patients inadequately controlled with metformin. In one of them the following results were found a year: From a mean baseline of 7.5%, HbA1c changes from baseline were -0.67% at week 52 in both groups, confirming non-inferiority. The proportions achieving an HbA1c < 7% were 63% (sitagliptin) and 59% (glipizide). Fasting plasma glucose changes from baseline were -0.56 mmol/L (-10.0 mg/dL) and -0.42 mmol/L (-7.5 mg/dL) for sitagliptin and glipizide, respectively<sup>[109]</sup>. With sitagliptin were observed less hypoglycaemia and less weight gain than with glipizide.

**Vildagliptin:** This is an iDPP4 which FDA was not approved so that is not being used in the United States. The usual dose is 50 mg twice daily when utilized as monotherapy, with metformin, or with a TZD, and 50 mg once daily (in the morning) when utilized with a sulfonylurea. No dose adjustment is necessary in patients with mild renal impairment (creatinine clearance  $\geq$  50 mL/min). In patients with moderate or severe renal impairment, the dose is 50 mg once daily.

In some studies comparing the efficacy and safety of vildagliptin compared with placebo target the treatment difference (vildagliptin-placebo) in adjusted mean change (AM Delta)  $\pm$  SE in HbA1c from baseline to endpoint it was  $-0.7\% \pm 0.1\%$  ( $P < 0.001$ ) and  $-1.1\% \pm 0.1\%$  ( $P < 0.001$ ) in patients receiving 50 or 100 mg of vildagliptin, respectively. The difference between treatments in the Delta GPA (GPA) was  $-0.8 \pm 0.3$  mmol/L ( $P = 0.003$ ) and  $-1.7 \pm 0.3$  mmol/L ( $P < 0.001$ ) in patients receiving 50 or 100 mg of vildagliptin, respectively<sup>[110]</sup>.

**Saxagliptin:** Saxagliptin is approved as a drug for home treatment of T2DM or dual therapy for patients not controlled with a sulfonylurea, metformin or TZD. The dose is 2.5 or 5 mg of saxagliptin once daily. The dose of 2.5 mg is recommended for patients with an eGFR  $\leq 50$  mL/min and patients taking drugs inhibitors of cytochrome P450 3A4/5 (e.g., ketoconazole), Saxagliptin monotherapy is effective, achieving reductions in HbA1c of 0.5 in naive patients vs placebo<sup>[111,112]</sup>. There are studies with saxagliptin (2.5, 5 and 10 mg) in dual therapy with metformin showed a statistically significant adjusted mean HbA1c decrease from baseline to week 24 compared to placebo ( $-0.59\%$ ,  $-0.69\%$ , and  $-0.58\%$  vs  $+0.13\%$ ; all  $P < 0.0001$ )<sup>[113]</sup>. There are also studies showing the efficacy of sitagliptin in combination with sulfonylureas and TZD.

**Linagliptin:** The dose of linagliptin is 5 mg once daily. It is eliminated mainly through the enterohepatic system so it is not necessary to adjust the dose in patients with renal or hepatic impairment. Inducers of CYP3A4 or P-glycoprotein (e.g., rifampicin) may reduce the effectiveness of this agent. In patients receiving these drugs should avoid the use of linagliptin.

In a monotherapy study vs placebo, linagliptin achieved a reduction in HbA1c of 0.44% against rising 0.25% with placebo in 6 mo<sup>[114]</sup>. In a 24 wk study in triple therapy in patients treated with metformin and sulfonylureas that was added linagliptin or placebo, appeared a reduction in HbA1c of 0.72% in the group with linagliptin vs 0.1% in the group with placebo<sup>[115]</sup>.

**Alogliptin:** The usual dose of alogliptin is 25 mg once daily, with dose reductions to 12.5 mg once daily in patients with creatinine clearance between 30 and 60 mL/min and to 6.25 mg daily in patients with creatinine clearance  $< 30$  mL/min or undergoing dialysis<sup>[116]</sup>.

In a study to twelve weeks in patients treated with metformin with poor control of their diabetes, alogliptin group achieved a reduction in HbA1c of 0.64% compared to an increase of 0.22% in the placebo group<sup>[117]</sup>. In another 26 wk studies, with alogliptin (12.5 or 25 mg once a day) vs placebo in patients with poorly controlled T2DM on a stable dose of glyburide ( $n = 500$ ) or insulin (alone or in combination with metformin,  $n = 390$ ) there were greater reductions in HbA1c in the alogliptin groups (mean change in HbA1c from baseline

$-0.39$ ,  $-0.53$  and  $+0.01$  percentage points for the 12.5, 25 mg, and placebo groups, respectively, in the glyburide trial, and  $-0.63$ ,  $-0.71$  and  $-0.13$  percentage points, respectively, in the insulin trial)<sup>[118,119]</sup>.

**Side effects:** These drugs are considered very safe since both the risk of hypoglycaemia and other adverse effects are rare. All of them at increased risk of hypoglycaemia in combination with sulfonylureas or insulin. In comparative studies have not observed any significant differences between them in the risk of hypoglycaemia. With vildagliptin and alogliptin have been reported cases of hepatic dysfunction unusually still advisable to monitor liver enzymes during the first three months of treatment. If an increase in transaminases of three times the upper limit of normal or greater persists, the drugs should be discontinued.

At present, there is insufficient data to know whether there is a causal relationship between acute pancreatitis and iDPP4<sup>[120-123]</sup>. They should be discontinued in patients with persistent severe abdominal pain. In patients with pancreatitis should not start these drugs, or if there is a history of this disease.

Commonly reported side effects include headache, nasopharyngitis, and upper respiratory tract infection<sup>[124,125]</sup>. Some, but not all, studies have reported a slight increased risk of gastrointestinal side effects with sitagliptin<sup>[108,109,126]</sup>.

**Cardiovascular effects:** Sitagliptin, saxagliptin and alogliptin have been studied for cardiovascular safety. They are TECOS, SAVOR-TIMI and EXAMINE studies respectively, with thousands of patients at high cardiovascular risk with a median follow up of 18 to 36 m.

In the TECOS study with sitagliptin 14735 patients with T2DM and established cardiovascular disease (history of major diseases of the coronary artery, ischemic cerebrovascular disease or peripheral arterial atherosclerotic disease) were randomized a group with sitagliptin and one with placebo, plus other diabetes medications (mainly metformin, sulfonylurea, insulin)<sup>[127]</sup>. After three years, the primary cardiovascular combined outcome (cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, or hospitalization for unstable angina) was observed in a similar proportion of diabetics (11.4% and 11.6% in the sitagliptin and placebo group's human resources, respectively, 0.98; 95%CI: 0.89-1.08). There was no significant difference in any of the individual components of the composite endpoint or the rate of hospitalization for heart failure (3.1% in each group).

In the test with saxagliptin (SAVOR-TIMI), 16492 patients with T2DM and either a history of cardiovascular disease or multiple risk factors for vascular disease were randomized to the branch of saxagliptin or placebo, and other medicines for diabetes (such as metformin, sulfonylureas, insulin). After a two-year follow-up, the first target (combination of cardiovascular death, nonfatal ischemic stroke or nonfatal myocardial

infarction) appeared in a similar number of diabetics in proportion, 7.3% and 7.2% in the saxagliptin and placebo, respectively; hazard ratio (HR) 1.00, 95%CI: 0.89-1.12<sup>[128]</sup>. Significantly more patients in the field of saxagliptin were hospitalized for heart failure (3.5% vs 2.8%; HR = 1.27, 95%CI: 1.07-1.51). It stresses significantly the hospitalization for heart failure in the saxagliptin study<sup>[129]</sup> increase. However, the possible association between heart failure and iDPP4 has been linked to other epidemiological data and claims data<sup>[130,131]</sup>.

In the EXAMINE trial alogliptin, 5380 patients with T2DM and either an acute myocardial infarction or unstable angina requiring recent hospitalization were randomized to alogliptin or placebo, along with other antidiabetic (mainly metformin, sulfonylureas, insulin)<sup>[132]</sup>. At 18 mo follow-up, the primary composite endpoint including cardiovascular death, nonfatal stroke, or nonfatal myocardial appeared in a very similar proportion of patients (11.3% and 11.8% in the branches of alogliptin and placebo respectively; HR 0.96, 95% of the unilateral CI: 1.16). In a post hoc analysis of the data, there was no significant difference in the rate of hospitalization for heart failure (3.1% and 2.9% in the branches of alogliptin and placebo, respectively; HR = 1.07, 95%CI: 0.79-1.46)<sup>[133]</sup>.

### **Sodium glucose co-transporter-2 inhibitor**

iSGLT2 inhibit renal reabsorption of glucose, increase its excretion and reduce hyperglycaemia in patients with T2DM. Therefore, reducing the reabsorption of glucose by inhibition of SGLT2 is a new way to treat T2DM. The increase in glucosuria and diuresis produced results in a reduction in weight and blood pressure<sup>[134]</sup>.

Kidneys from healthy people filter approximately 180 g of glucose each day through renal glomerulus and reabsorbed in the then proximal convoluted tubule. This is possible by passive and active co-carriers which are known as glucose transporter (GLUT) and SGLT<sup>[135]</sup> conveyors. There are two types of SGLT; SGLT1 located mainly in the small intestine and the kidney proximal convoluted tubule, and SGLT2 located only in the proximal tubule (segment 1 and 2), that are responsible for about 90% of glucose reabsorption<sup>[7]</sup>. The other 10% of the glucose is reabsorbed by SGLT1 in segment 3. SGLT2 inhibitors block the SGLT2 transporter in the proximal tubule, to lower glucose reabsorption and increase its excretion in the urine. Glucose is excreted in the urine and plasma levels are reduced by improving glycaemia figures plasma<sup>[136-138]</sup>. It is an independent mechanism of insulin, there is low risk for hypoglycaemia, and no risk of fatigue or overstimulation of the beta cells<sup>[139]</sup>. Due to its mode of action is based on normal glomerular-tubular function; the iSGLT2 efficiency is lower in patients with renal failure<sup>[140]</sup>. The three most representative drugs family iSGLT2 are: Dapagliflozin, canagliflozin and empagliflozin.

**Dapagliflozin:** Dapagliflozin was the first iSGLT2

employee, and has many published data from clinical trials. In phase 3 trials comparing placebo for 24 wk and dapagliflozin (2.5, 5 and 10 mg once daily) used alone or added to metformin<sup>[141]</sup>, pioglitazone<sup>[142]</sup>, glimepiride<sup>[143]</sup> or insulin<sup>[144]</sup> was observed that HbA1c and fasting plasma glucose in patients with T2DM was reduced. In tests longer-term (102 wk) added to metformin, dapagliflozin resulted in a sustained decrease in HbA1c, glucose fasting blood glucose and weight without increasing the risk of hypoglycaemia in patients with T2DM not controlled on metformin alone<sup>[145]</sup>. The initial decrease in HbA1c observed at 24 wk with both doses of dapagliflozin (5 or 10 mg) added to metformin was maintained at 102 wk, and was superior to placebo (-0.58%, -0.78% and 0.02% against). Also the low fasting plasma glucose with both doses of dapagliflozin, remained and was higher than placebo (-1.47 mmol/L and -1.36 mmol/L vs -0.58 mmol/L). This drug has studies which compared with patients whose hyperglycaemia glipizide was poorly controlled by metformin<sup>[146]</sup>. After 52 wk, a drop in HbA1c starting from the baseline of -0.52% is target with dapagliflozin ( $\leq$  10 mg/d) and glipizide ( $\leq$  20 mg/d). Weight reduction was greater with dapagliflozin (-3.2 kg) vs glipizide (+1.4 kg). Dapagliflozin ( $\leq$  10 mg/d) in T2DM patients was non-inferior to glipizide ( $\leq$  20 mg/d) in reduction of HbA1c at 52 wk (both -0.52%). At 4 years the HbA1c reduction is attenuated in both groups, but more in the glipizide vs dapagliflozin (+0.2% vs -0.1%). There were differences in weight change, with weight loss in the dapagliflozin group vs weight gain in the glipizide group (-3.95 kg vs +1.12 kg). In the dapagliflozin group decreases the mean average of systolic blood pressure, but did no change in the glipizide group (difference: -3.7 mmHg)<sup>[146]</sup>.

**Canagliflozin:** Canagliflozin was the first of this family of drugs approved by the FDA and began its commercialization in March 2013 for use in T2DM. It is an effective drug in monotherapy and after 26 wk of treatment with canagliflozin 100 mg and 300 mg once daily significantly reduced HbA1c (-0.77% and -1.03% respectively) in patients with T2DM not controlled with diet and exercise compared to placebo (0.14%,  $P < 0.001$ )<sup>[147]</sup>. Also, significantly reduced fasting blood glucose, -27 mg/dL to -34 mg/dL with both doses of canagliflozin (placebo = 9 mg/dL,  $P < 0.001$ ). Get for this reason a larger number of patients in target HbA1c  $< 7.0\%$  compared to placebo (44.5% to 62.4% vs 20.6%;  $P < 0.001$ ). At week 52 in the double therapy, 300 mg canagliflozin under more HbA1c that sitagliptin (-0.73%, -0.88%, -0.73%, respectively)<sup>[148]</sup>. Data reduction in body weight with canagliflozin 100 and 300 mg vs placebo at week 26 were -3.7, -4.2, -1.2 kg, respectively ( $P < 0.001$ ) and vs sitagliptin at week 52 were -3.8, -4.2, -1.3 kg, respectively ( $P < 0.001$ ). Also, in combination therapy, improved canagliflozin reducing body weight, HbA1c, and tolerance was better than in diabetics treated with metformin plus



sulfonylurea more than 52 wk<sup>[149]</sup>. At week 26, HbA1c decreased significantly with canagliflozin 100 and 300 mg vs placebo (-0.85%, -1.06%, -0.13%;  $P < 0.001$ ); this improvement was maintained at week 52 (-0.74%, -0.96%, +0.01%). Both doses of canagliflozin (100 mg/d and 300 mg/d) showed non-inferiority in HbA1c reduction (-0.82% and -0.93%) compared to glimepiride for 52 wk of treatment in diabetic subjects treated with metformin. Canagliflozin 300 mg/d was more effective than glimepiride in decreasing HbA1c, and both doses of canagliflozin were higher than glimepiride in lowering body weight (-3.7 kg to 100 mg/d, -4.0 kg with 300 mg/d vs +0.7 kg with glimepiride)<sup>[149]</sup>. Data from this study, objectified to 104 wk, showed that reductions in HbA1c remained with canagliflozin 100 and 300 mg and glimepiride vs placebo at week 104 (-0.65%, -0.55% and -0.76%), and both canagliflozin dose were better than glimepiride in weight reduction (-4.1 kg with 100 mg/d, -4.2 kg with 300 mg/d vs +0.9 kg with glimepiride)<sup>[150]</sup>.

**Empagliflozin:** Empagliflozin is a drug that has eight multinational clinical trials, including a very important safety trial of cardiovascular risk. Data empagliflozin 12 wk at doses 5-25 mg/d are increased excretion of glucose and a decrease of fasting blood glucose (-31.1 mg/dL at 25 mg vs an increase +0.8 mg/dL placebo), HbA1c (-0.63% vs 25 mg vs an increase of +0.09%) and body weight (-2.0 kg to 25 mg vs -0.8 kg) in T2DM<sup>[151]</sup>. Both doses of empagliflozin (10 mg or 25 mg daily) added to metformin received greater reductions in HbA1c vs sitagliptin (-0.34% to -0.63% vs -0.40%) and these were maintained for 90 wk. The fasting glucose reduction was also higher after 90 wk of treatment with two doses of empagliflozin against sitagliptin (-21 mg/dL and -32 mg/dL vs -16 mg/dL), and these effects were maintained over the treatment period<sup>[152]</sup>. The weight was reduced from the baseline of -2.2 to -4.0 kg with empagliflozin, -1.3 kg with metformin, and sitagliptin -0.4 kg after 90 wk<sup>[153]</sup>. In a randomized, double-blind empagliflozin (10, 25 mg) or placebo add-on to basal insulin for 78 wk; compared with placebo, 10 and 25 mg/d of empagliflozin significantly lower body weight (-2.2 kg, -2.0 kg, and +0.7 kg respectively), and decreased HbA1c (-0.48%, -0.64%, and -0.02%, respectively), and systolic blood pressure (-4.1 mmHg, -2.4 mmHg, and +0.1 mmHg, respectively)<sup>[154]</sup>. Therefore, a long-term empagliflozin is an effective treatment for patients with T2DM.

**Pleiotropic effects:** iSGLT2 achieve a decrease in body weight between 1-5 kg medium<sup>[155]</sup>. Weight loss is greater if, in addition, the use of these drugs able to decrease the dose of insulin. Patients fastest achieve greater weight reduction<sup>[156]</sup>. The results of studies over 4 years in T2DM patients treated with dapagliflozin vs glimepiride, both in combination with metformin, showed a reduction of 3.65 kg in the dapagliflozin group compared with the branch of glimepiride that gained an

average of 0.73 kg<sup>[155]</sup>. There has been demonstrated in multiple studies that the loss of weight produced by these medicaments is principally secondary to a loss of fat mass (especially visceral abnormal fat) and not due to a volume depletion. Also, one has found a reduction of the abdominal perimeter<sup>[154]</sup>. In studies with canagliflozin it was observed that the 0.66% reduction in body weight was fat mass, and 0.33% was lean body mass. The association of iSGLT2 with anti-diabetic drugs that increase the weight (pioglitazone, insulin) can get minimize this gain<sup>[144]</sup>. iSGLT2 also reduce the systolic (-1.66 mmHg to -6.9 mmHg) and diastolic (-0.88 mmHg to -3.5 mmHg) blood pressure. This decrease occurs because the initial osmotic diuresis, and subsequent inhibition of the renin-angiotensin system<sup>[157]</sup>, and the decrease is independent from the levels of glucose or from the weight of the patients. Also the effects on blood pressure were not dose-dependent and were not accompanied by any notable changes in heart rate or increases in hypotension and/or syncope<sup>[158,159]</sup>. Some analysis from phase IIb studies with empagliflozin revealed even greater decreases in systolic blood pressure of 13.4 mmHg to 17 mmHg amongst a subgroup of patients with a baseline systolic blood pressure > 140 mmHg compared to the overall population. In a study of dapagliflozin it was that the effects on blood pressure were more important in patients with a baseline systolic blood pressure > 140 mmHg.

It's not clear the effect of these medicaments on the lipid profile. The same results do not exist with all the iSGLT2. In some studies are lipid-friendly and in others are lipid-neutral drugs. Canagliflozin, for example, increases HDL cholesterol by 7.1%, LDL cholesterol by 7.1%, and reduces triglycerides by 2.3%, over 52 and 104 wk<sup>[160]</sup>. These modifications in lipid profiles were not observed with other iSGLT2 such dapagliflozin<sup>[161]</sup>.

This new drugs also have a paper reducing the serum uric acid levels. They can decrease the levels in a range from -5.9% to -17.8% with the effect sustained for 2 years<sup>[162]</sup>.

Finally, SGLT2 is associated with glomerular hyperfiltration; thus blockade of SGLT2 has potential nephroprotective action<sup>[163]</sup>.

**Side effects:** The iSGLT2 has a similar incidence of adverse events in clinical trials which are given with other oral antidiabetic agents. The overall incidence of adverse events moves between 57.3% to 83.0%, and serious adverse events is between 1.0% and 12.6%<sup>[155]</sup>.

Increased glucosuria produces the urogenital tract infections that are the most common side effects of these drugs<sup>[164]</sup>, especially in women and uncircumcised men. Genital mycotic infections in women were vulvovaginal candidiasis, vulvitis, vulvovaginitis, and vulvovaginal mycotic infection. In male patients balanitis and balanoposthitis occur. In trials with dapagliflozin 2.5, 5 and 10 mg doses, the incidence of urogenital tract infections was 4.1%; 5.7% and 4.8% depending on the

dose of the drug vs 0.9% in placebo patients<sup>[165]</sup>.

Another adverse effects of these agents also derived from his mechanism of action is the orthostatic hypotension and the volume depletion. These drugs are associated with an osmotic diuresis that can produce it. In randomized controlled trials the occurrence of these side effects was very low (< 3%)<sup>[166]</sup>. The extra diuresis experienced per day does not cause nocturia<sup>[167]</sup>.

iSGLT2 have a non-insulin based mechanism and because of that the risk of hypoglycaemia is minimal with them. This risk can increase in therapy combined with sulfonylureas or insulin.

The use of iSGLT2 is associated with changes in bone turnover markers, with reduction in bone formation without changes in bone mineral density. There are long-term studies do not confirm these changes related to skeletal system<sup>[150,154]</sup>. A 2-year study with dapagliflozin, no objective changes in bone turnover markers compared with placebo when combined with metformin<sup>[162]</sup>.

There have been reports of euglycaemic ketoacidosis in some patients treated with iSGLT2<sup>[168]</sup>. They are studying the mechanisms by which this complication may occur. This is frames ketoacidosis with blood glucose levels < 200 mg/dL. The possible cause of the euglycaemic ketoacidosis can be attributed to the recent use of insulin, reducing calorie intake, alcohol abuse, chronic liver disease and glycogen storage disorders<sup>[169]</sup>.

**Cardiovascular effects:** All iSGLT2 have launched important studies of cardiovascular safety. It has now ended with empagliflozin conducted with promising results for this therapeutic group.

EMPA-REG is an international prospective, placebo-controlled trial of empagliflozin cardiovascular outcomes in patients with T2DM and know cardiovascular disease. In the trial he managed to reach the main objective of non-inferiority and also showed, after 3.1 years of median follow-up, the superiority of empagliflozin group (10 or 25 mg/d) vs placebo in what with respect to the primary composite cardiovascular endpoint (-14%), hospitalizations for heart failure (-35%), cardiovascular mortality (-38%) and mortality from all causes (-32%, each  $P < 0.001$ ). The decrease in mortality appeared from early stages (< 6 mo) and referred to all subgroups, without any apparent heterogeneity. These reductions in mortality appear to be related to the diuretic and natriuretic effect of empagliflozin, and not with concomitant reductions in HbA1c, body weight, blood pressure, waist circumference and serum uric acid levels in the field of empagliflozin respect to placebo. Tolerance and safety of empagliflozin was good, objectifying only a moderate increase in benign genital fungal infections, adverse event known iSGLT2<sup>[170]</sup>.

## INJECTABLE AGENTS

### RA-GLP1

Human GLP1 is secreted in response to food intake and

stimulates insulin release<sup>[171]</sup>. Two incretins have been identified: GLP1, which is produced and released mainly by L-cells located in the distal ileum and GIP, which is secreted by enteroendocrine K-cells in the proximal gut.

GLP1 treatment in T2DM patients increased insulin secretion glucose dependent and decrease secretion of glucagon, slowed gastric emptying, raised satiety, and reduce food intake<sup>[172]</sup>. GLP1 also protect against myocardial ischemia<sup>[173,174]</sup>. In blood vessels promotes endothelium-independent artery relaxation protecting against endothelial dysfunction. Also have effect in protecting renal function by increasing diuresis and natriuresis<sup>[175,176]</sup>. All of these actions allow lower blood pressure and have positive effects on cardiovascular risk markers such as plasminogen activator inhibitor and brain natriuretic peptide.

The use of GLP1 therapy is limited by its rapid breakdown by DPP4; it has a short half-life: 1-2 min. Multiple RA-GLP1 have been developed with the physiological effects of GLP1 and an extended duration of action. RA-GLP1 agonists have proven efficacy for lowering HbA1c, fasting plasma glucose, body weight and systolic blood pressure, with a reduced risk of hypoglycaemia<sup>[6]</sup>. EASD/ADA and AACE guidelines recommended their use in combination with metformin, or as triple therapy in combination with metformin, sulfonylureas, TZD or insulin<sup>[9,42]</sup>.

RA-GLP1 are classified by their duration in short-acting or long-acting. Short acting RA-GLP1 are exenatide twice daily and lixisenatide; their provide short-lived GLP1 receptor activation; tend to have a more accentuated effect on postprandial hyperglycaemia and gastric emptying and less effect on fasting glucose. Long acting RA-GLP1 are liraglutide, once-weekly formulation of exenatide Exenatide LAR), albiglutide and dulaglutide; they activate the GLP1 receptor continuously, compared with short-acting effect on gastric emptying and postprandial glucose. Exenatide, exenatide LAR and lixisenatide derived from the exendin-4 molecule, a peptide with a 53% homology with human GLP1<sup>[177-179]</sup>. Liraglutide, albiglutide and dulaglutide are 97%, 95% and 90% identity.

**Exenatide:** Exenatide was the first RA-GLP1 to be approved for glycaemic control. Is a synthetic 39-amino acid peptide identical to the exendin-4 molecule isolated from salivary glands of the Gila monster; shares approximately 53% homology with native GLP1. The usual dose is 5-10 µg twice-daily subcutaneous injection.

Exenatide in monotherapy lowered HbA1c by 0.7%-0.9% and fasting plasma glucose by 17.5-18.7 mg/dL. The efficacy and safety of exenatide has been proved in several clinical studies<sup>[180-183]</sup>. Up to 46% of patients treated with exenatide achieved HbA1c ≤ 7% objective compared with up to 13% of placebo group. Moreover, mean change in body weight from baseline was greater in the exenatide group (-1.6 to -2.8 kg) than in the placebo group (-0.3 to -0.9 kg)<sup>[180-182]</sup>. When

compared exenatide with insulin glargine or biphasic insulin aspart in patients with T2DM not controlled with oral agents, there were similar reductions in HbA1c in the exenatide and insulin groups (approximately -1.0%) suggesting non-inferiority of exenatide compared to insulin in relation to HbA1c reduction<sup>[184,185]</sup>. Exenatide showed weight loss and reduction in postprandial glycaemia compared with any insulin therapy, and lower rate of nocturnal hypoglycaemia compared with insulin glargine. In the glargine comparison study, insulin was titrated based upon achieving a target fasting glucose level < 100 mg/dL (5.6 mmol/L).

**Lixisenatide:** Lixisenatide is a RA-GLP1 that shares some structural elements with exendin-4. Compared with native GLP1, it has a prolonged half-life (2.7 to 4.3 h). Is available in Europe, not in United States, for use in combination with oral agents or insulin; is not considered a first-line therapy. Is available in a prefilled pen containing 14 doses of 10 or 20 mcg of lixisenatide. The initial dose is 10 mcg subcutaneously once daily within one hour prior to any meal of the day; after 2 wk the dose can be increased to 20 mcg.

Lixisenatide has been studied as monotherapy and in combination with one or two oral agents (metformin, pioglitazone, sulfonylureas). In a 24-wk double-blind trial of lixisenatide 20 mcg once daily vs placebo in 680 T2DM patients inadequately controlled with metformin (mean HbA1c 8.1%), the mean reduction in HbA1c was significantly greater with lixisenatide (-0.9% vs -0.4%)<sup>[186]</sup>, and in another 24-wk no inferiority trial of once-daily subcutaneous lixisenatide 20 mcg once daily vs exenatide 10 mcg twice daily in 634 T2DM patients inadequately controlled with metformin alone (mean baseline HbA1c 8%), lixisenatide was no inferior to exenatide (mean change HbA1c -0.79% vs -0.96% with exenatide)<sup>[187]</sup>.

Lixisenatide has been also used in combination with basal insulin therapy<sup>[188-190]</sup>. In a 24-wk double-blind trial, in 495 patients with T2DM not controlled with insulin glargine and metformin (mean HbA1c 8.4%), HbA1c reduction was significantly greater in the lixisenatide group compared to placebo (-0.6% vs -0.3%).

**Liraglutide:** Liraglutide is a human RA-GLP1, obtained through modifications of the human GLP1, with a large half life, which is administered once a day. Is available for use as monotherapy (adjunct to life style changes) or in combination with oral agents and basal insulin in adults with T2DM. The initial dose is 0.6 mg once daily subcutaneously the first week; and after the dose should be increased to 1.2 mg; and if HbA1c remain above the goal range the dose can be increased to 1.8 mg. It can be administered at any time of the day, with or without meals.

In clinical studies, administration of liraglutide (0.6-1.8 mg/d), alone or added to other antidiabetics agents, resulted in a reduction in HbA1c between 0.6%-1.6%. In a 52-wk trial of monotherapy with

liraglutide (1.2 or 1.8 mg) vs glimepiride (8 mg) in 746 patients with recently diagnosed T2DM, the proportions of patients achieving an HbA1c  $\leq$  7% were 43%, 51% and 28%, respectively. Reductions in HbA1c were significantly greater with liraglutide 1.2 and 1.8 mg (-0.84% and -1.14% vs -0.51% with glimepiride). In addition, the HbA1c reduction with liraglutide (1.8 mg) was higher than that with other doses<sup>[191]</sup>. In another 26-wk double-blind trial, 413 T2DM patients not controlled with basal insulin and metformin were randomly assigned to exchange basal insulin with insulin degludec or insulin degludec plus liraglutide; all patients continued metformin<sup>[192]</sup>. The reduction in HbA1c was significantly greater in the degludec-liraglutide group (treatment difference -1.1%). The mean reduction in weight with degludec-liraglutide was 2.7 kg vs no change with degludec alone.

**Exenatide LAR:** Administration of exenatide LAR was proved more effective than highest dose of exenatide twice-daily<sup>[193,194]</sup>, sitagliptin and pioglitazone<sup>[195]</sup>, and insulin glargine<sup>[196]</sup> in T2DM patients treated with oral hypoglycaemic agents. Is available for use as adjunct to lifestyle changes to improve glycaemic control in T2DM. The usual dose is 2 mg subcutaneously once weekly at any time of the day with or without meals.

**Albiglutide:** It is a RA-GLP1 with a half-life of five to seven days, which allows once-weekly administration. It is available for use as monotherapy or in combination with oral agents or basal insulin. Is available in prefilled pen that contain a powder (30 or 50 mg), and a diluent to make a solution that is injected subcutaneously once weekly. The initial dose is 30 mg, and if after 6-8 wk blood glucose remain above the goal, the dose can be increased to 50 mg.

Albiglutide has been studied as monotherapy and in combination with one or two oral agents (metformin, pioglitazone, sulfonylureas and insulin). As examples: In a one-year trial of albiglutide vs insulin glargine in 779 T2DM patients inadequately controlled with metformin (with or without a sulfonylurea), the mean HbA1c reduced from 8.28% to 7.62% in the albiglutide group and from 8.36% to 7.55% in the glargine group<sup>[197]</sup>. Albiglutide met its pre-specified non-inferiority margin; however the comparison should be interpreted with caution because the dose of glargine was not systematically up titrated. Glargine was significantly more effective than albiglutide in reducing fasting blood sugar. In another two-year trial of weekly albiglutide vs daily sitagliptin, daily glimepiride, and weekly placebo in patients with T2DM inadequately controlled with metformin (mean HbA1c 9.1% to 8.2%), the reduction in HbA1c from baseline among the four groups was -0.6%, -0.3%, -0.4%, and +0.3%, respectively<sup>[198]</sup>. Although statistically significant, the mean reduction in HbA1c from baseline in the albiglutide group compared with the sitagliptin and glimepiride groups was small and of uncertain clinical relevance.

**Dulaglutide:** It is the last RA-GLP1 in appear. It has a structure that gives it the properties of slow absorption and reduced renal clearance rate. It is available for use as monotherapy or in combination with oral agents or insulin, in a ready-mixed pen at dose of 0.75 mg in monotherapy once weekly or 1.5 mg in combination, once weekly.

It has been compared with other antiabetic agents such metformin, iDPP4, insulin and other RA-GLP1, with a reduction in HbA1c ranging from -0.78% to -1.51%. In a 52-wk trial of weekly dulaglutide (0.75 or 1.5 mg weekly) vs sitagliptin in 1098 T2DM patients not controlled with metformin, the reduction in mean HbA1c was significantly greater with either dose of dulaglutide (mean HbA1c reduced from 8.2% to 7.3% with dulaglutide 0.75 mg weekly, from 8.1% to 7.0% with dulaglutide 1.5 mg weekly, and from 8% to 7.6% with sitagliptin)<sup>[199]</sup>. The mean change in body weight was significantly better with dulaglutide (-2.6 kg and -3 kg vs -1.53 kg with sitagliptin).

**Precautions and side effects:** All RA-GLP-1 should not be used in patients with history of pancreatitis and are not approved for use in T1DM. Exenatide and lixisenatide should not be utilized in patients with an eGFR < 30 mL/min and with severe gastrointestinal disease. Liraglutide, albiglutide and dulaglutide should not be used in patients with personal or family history of medullary thyroid cancer or multiple endocrine neoplasia 2A or 2B.

The mayor side effect are gastrointestinal, particularly nausea, vomiting and diarrhoea. It appears with lower frequency with exenatide LAR or lixisenatide than exenatide twice daily; albiglutide had lower rates than liraglutide and liraglutide and dulaglutide are similar. The risk of hypoglycaemic events is small, and may occur when RA-GLP-1 is given in conjunction with other treatments that cause hypoglycaemia, e.g., basal insulin, sulfonylureas.

Injection site reactions are more common with RA-GLP-1 than with insulin. Between RA-GLP1 are more common with exenatide LAR and with albiglutide. These reactions can be abscess, cellulitis and necrosis with or without subcutaneous nodules. Antibodies to RA-GLP1 may occur. In the majority of patients, the titre of antibodies decreases over time and does not affect glycaemic control. In a meta-analysis of 17 trials, the proportion of patients with antibodies against RA-GLP1 was higher in the albiglutide group compared with placebo (6.4% albiglutide 30 mg weekly vs 2% with placebo)<sup>[200]</sup>.

**Head-to-head comparisons of RA-GLP1:** They have been published 9 phase III clinical trials, comparing different pairs of RA-GLP1<sup>[201]</sup>. One of them is with taspoglutide: T-emerge 2; we are not going to include it in the present review because its development was halted because of serious hypersensitivity reactions and gastrointestinal adverse events.

DURATION-1<sup>[193]</sup>: Exenatide twice daily vs exenatide

LAR. Duration: 30 wk. Inclusion criteria:  $\geq 16$  years, therapy with lifestyle changes, or with 1-2 oral agents (metformin, sulfonylureas and/or TZD), HbA1c 7.1%-11.0%, fasting plasma glucose < 16 mmol/L, and body mass index (BMI) 25-45 kg/m<sup>2</sup>.

DURATION-5<sup>[194]</sup>: Exenatide twice daily vs exenatide LAR. Duration 24 wk. Inclusion criteria:  $\geq 18$  years, therapy with lifestyle changes, or with metformin, sulfonylureas, TZD or a combination, HbA1c 7.1%-11.0%, fasting plasma glucose < 15.5 mmol/L, and BMI 25-45 kg/m<sup>2</sup>.

DURATION-6<sup>[202]</sup>: Exenatide LAR vs liraglutide once daily. Duration 26 wk. Inclusion criteria:  $\geq 18$  years, therapy with lifestyle changes and oral agents (metformin, sulfonylureas, metformin + sulfonylureas or metformin + pioglitazone), HbA1c 7.1%-11.0%, and BMI  $\leq 45$  kg/m<sup>2</sup> and stable body weight.

LEAD-6<sup>[203]</sup>: Exenatide twice daily vs liraglutide once daily. Duration 26 wk. Inclusion criteria: 18-80 years, treated with metformin, sulfonylureas or both, HbA1c 7.0%-11.0%, and BMI  $\leq 45$  kg/m<sup>2</sup>.

GetGoal-X<sup>[188]</sup>: Exenatide twice daily vs lixisenatide once daily. Duration 24 wk. Inclusion criteria: 21-84 year, therapy with metformin, and HbA1c 7.0%-11.0%.

HARMONY 7<sup>[204]</sup>: Albiglutide once weekly vs liraglutide once daily. Duration 32 wk. Inclusion criteria:  $\geq 18$  years, therapy with metformin, sulfonylureas, TZD or a combination, HbA1c 7.0%-10.0%, and BMI 20-45 kg/m<sup>2</sup>.

AWARD-6<sup>[205]</sup>: Dulaglutide once weekly vs liraglutide once daily. Duration 26 wk. Inclusion criteria:  $\geq 18$  years, therapy with metformin, and HbA1c 7.0%-10.0%.

Kapitza *et al.*<sup>[206]</sup>: Lixisenatide once daily vs liraglutide once daily. Duration 28 wk. Inclusion criteria: 37-74 years, therapy with metformin, and HbA1c 6.5%-9.0%.

Effects on HbA1c: In the DURATION-1 and DURATION-5 exenatide LAR produced more consistent and greater reductions in HbA1c than exenatide twice daily. In the GetGoal-X exenatide twice daily showed greater HbA1c reduction than lixisenatide. Liraglutide in LEAD-6 and DURATION-6 reach greater HbA1c reductions than exenatide twice daily or exenatide LAR, and in HARMONY 7 shows also greater reductions than albiglutide. Liraglutide and dulaglutide did not differ in AWARD-6 study.

Effects on weight: It varies among RA-GLP1 and studies. In DURATION-1 and DURATION-5, there were no significant differences in weight loss between the two exenatide preparations. In LEAD-6, liraglutide and exenatide twice daily loss similar weight as in GetGoal-X study, between exenatide twice daily and lixisenatide, the difference was non-significant. Only in AWARD-6 and in the study by Kapitza *et al.*<sup>[206]</sup>, liraglutide revealed significantly greater reductions than dulaglutide and lixisenatide.

Cardiovascular effects: Improvements in both systolic and diastolic blood pressure have been reported in clinical trials; however in these head-to-head trials there were no statistically significant differences between treatments. Increases in resting heart rate have been reported. With exenatide twice-daily the



**Table 5** Type of insulin by onset of action, peak effect and duration of action

Insulin type	Onset of action	Peak effect	Duration of action
Lispro, aspart, glulisine	5 to 15 min	45 to 75 min	2 to 4 h
Regular	About 30 min	2 to 4 h	5 to 8 h
NPH	About 2 h	4 to 12 h	18 to 28 h
Insulin glargine	About 2 h	No peak	20 to 24 h
Insulin detemir	About 2 h	No peak	6 to 24 h <sup>1</sup>
NPL	About 2 h	Six hours	15 h
Insulin degludec	About 2 h	No peak	> 40 h
Insulin U-300	About 2 h	No peak	> 36 h

<sup>1</sup>Duration of action is dose-dependent. At higher doses ( $\geq 0.8$  units/kg), mean duration of action is longer and less variable. Modified from: McCulloch DK. General principles of insulin therapy in diabetes mellitus. Uptodate, March 24, 2016. Available from: URL: <http://www.uptodate.com/contents/general-principles-of-insulin-therapy-in-diabetes-mellitus>.

heart rate increases, but it is lower than with exenatide LAR or liraglutide; dulaglutide is similar to liraglutide. With lixisenatide and albiglutide has not shown an increase in heart rate.

### Insulin

Insulin is utilized in the treatment of patients with all types of diabetes<sup>[207]</sup>. Human insulin preparations (NPH and regular insulin) do not imitate endogenous insulin secretion (basal and postprandial). Then, insulin analogues (aspart, lispro, glulisine, detemir, glargine, degludec and U-300) were developed. They have increased the flexibility and efficacy of diabetes management. The very rapid-acting insulin analogs have both: Faster and shorter duration of action than regular insulin for pre-meal coverage, while the long-acting analogs have a longer duration of action allowing once-daily dosing; also shows less day-to-day variability<sup>[208]</sup> (Table 5).

**Insulin preparations:** Long-acting insulins (glargine and detemir), and ultra-long-acting insulins (degludec and Glargine U-300) can be combined with rapid-acting insulins (aspart, lispro or glulisine) in basal bolus therapy.

Insulin glargine and human insulin are the same except for a substitution of glycine for asparagine in position A21 and by the addition of two arginine molecules in the B-chain of the insulin molecule<sup>[209]</sup>. These modifications originate a change in the pH such that, after administration, glargine precipitates in the subcutaneous tissue making hexamers, which delays absorption and extends duration of action. Glargine has a duration of action that usually lasts 24 h. Glargine cannot be mixed with rapid-acting insulins as the kinetics of both the rapid acting insulin and glargine and will be modified.

Insulin detemir is another insulin analog developed by removing a threonine and acylating a lysine with

14-carbon fatty acid; the fatty acid side chain allows albumin binding and results in prolongation of action. Clinical trials in patients with type 1 diabetes have suggested that twice-per-day injections may be necessary to achieve acceptable basal rate coverage and optimal glycaemic control<sup>[210]</sup>. In T2DM, where endogenous insulin secretion may mask any deficiencies in basal insulin, the data are less clear. Nevertheless the duration of action is dose-dependent; at higher doses mean duration of action is longer. Detemir cannot be mixed with rapid-acting insulins.

When glargine and detemir are administered in high doses, both show a peak on pharmacokinetic and pharmacodynamics profile<sup>[211]</sup>; also there is still interindividual variability and low doses are insufficient to cover a 24-h period<sup>[212]</sup>. Therefore, new ultra-long insulins were developed: Degludec, glargine U300 and LY2605541 (PEGylated Lispro). Lilly had discontinued the development of the last one because of hepatic lipid accumulation.

Insulin degludec is a modified B chain analogue that forms hexamers and di-hexamers when is administered. Compared with other long-acting insulins (glargine and detemir), the insulin degludec profile is flatter with a half life greater than 25 h, and action that exceeds 42 h, which results in a reduction of confirmed and nocturnal hypoglycaemias<sup>[213]</sup>. Glargine U300 is the same glargine molecule concentrated three times; so it has the same mechanism to slow its absorption as insulin glargine.

At present there are no head-to-head comparisons of insulin degludec and Glargine U-300. We are going to analyse clinical trials of both of them but comparisons between them should not be made because the studies are different: for example hypoglycaemia definition use in degludec is plasma glucose threshold of 3.1 mmol/L (55.85 mg/dL) and in Glargine U-300 is 3.9 mmol/L (70.26 mg/dL).

**Clinical trials in T2DM:** BEGIN basal-bolus type 2 study is a 52-wk, randomised, treat-to-target, parallel-group, open-label, non-inferiority trial. Compared the efficacy and safety of once-daily insulin degludec with once-daily insulin glargine in a basal-bolus regimen with mealtime insulin aspart, with or without metformin, pioglitazone, or both in participants with T2DM<sup>[214]</sup>. After 1 year, HbA1c decreased by 1.1% in the degludec group and 1.2% in the glargine group. Rates of overall confirmed hypoglycaemia (plasma glucose < 55 mg/dL) were lower with degludec, as well as rates of confirmed nocturnal hypoglycaemia. These results were maintained in a 26-wk extension of this study with fewer hypoglycaemic episodes (24% overall reduction and 31% confirmed nocturnal episodes reduction)<sup>[215]</sup>.

BEGIN Once Long was a 1 year phase 3 trial with type 2 insulin naive patients not controlled with oral hypoglycaemic agents. Again insulin degludec shows non inferiority in reducing HbA1c, and demonstrate a lower rate of nocturnal hypoglycaemia compared with

glargine<sup>[216]</sup>.

EDITION 1, 2 and 3<sup>[217-219]</sup> evaluated Gla-U300 in T2DM patients through 6 mo. The primary endpoint in the three studies was meeting the non-inferiority criterion in reduction HbA1c levels; which is confirmed in all studies; and the secondary endpoint was the percentage of patients with one or more confirmed or several nocturnal hypoglycaemias between week 9 and month 6. In EDITION 1 fewer patients reported one or more confirmed ( $< 3.9$  mmol/L or  $< 70$  mg/dL) or severe nocturnal hypoglycaemic events between week 9 and month 6 with Gla-U300 [36% vs 46% with Gla-U100; relative risk 0.79 (95%CI: 0.67-0.93);  $P < 0.005$ ]. In EDITION 2, again the percentage of patients with nocturnal hypoglycaemia was lower in those with Gla-U300 than with Gla-U100 with a risk reduction of 23%. In EDITION 3, the percentage of patients with nocturnal hypoglycaemia was statistically similar in patients with Gla-U300 and Gla-U100.

In conclusion, insulin degludec showed similar efficacy in reducing HbA1c to insulin glargine, with a decreased risk of confirmed and nocturnal hypoglycaemia.

## OTHER TREATMENTS

### Colesevelam

Colesevelam is a bile acid sequestrant that reduces LDL cholesterol in patients with hypercholesterolemia.

**Mechanism of action:** Possibly colesevelam interferes glucose absorption at gastrointestinal level.

**Efficacy:** In T2DM patients not controlled, colesevelam added to treatment of oral hypoglycaemic agents or insulin resulted in a reduction of HbA1c levels of 0.5%<sup>[220-222]</sup>.

**Side effects:** nausea, constipation and dyspepsia are frequent side effects. Also increases triglyceride concentrations by approximately 20%. We do not recommend colesevelam to treat T2DM patients due the modest glucose-lowering effectiveness, expense, and limited clinical experience.

### Bromocriptine

Bromocriptine is a dopamine agonist that has been used for the treatment of hyperprolactinemia and Parkinson disease.

**Mechanism of action:** The mechanism of action in reducing blood glucose is unknown. A quick release formulation of bromocriptine (Cycloset) was approved by the FDA for the treatment of T2DM<sup>[223]</sup>.

**Efficacy:** In short-term clinical trials in T2DM patients, bromocriptine (up to 4.8 mg daily) as monotherapy or added to sulfonylureas reduce HbA1c compared with placebo in 0.4%-0.5%<sup>[223,224]</sup>.

**Side effects:** Nausea, vomiting and headache<sup>[225]</sup> are frequent side effects. We do not recommend bromocriptine to treat T2DM patients due its glucose lowering effect and very frequent side effects.

### Pramlintide

Pramlintide is an amylin analog that is administered by mealtime subcutaneous injection. It is available for use for both T1 and insulin-treated T2DM; is only be used in patients also taking prandial insulin. Pramlintide replicates amylin actions and controls glucose without causing weight gain.

**Mechanism of action:** Pramlintide control postprandial blood glucose levels by slowing gastric emptying, promoting satiety, and reducing the postprandial glucagon increase in patients with diabetes<sup>[226]</sup>. The effects are glucose-dependent. Pramlintide does not cause hypoglycaemia in the absence of therapies that may cause hypoglycaemia. Supraphysiologic doses of pramlintide do not provoke hypoglycaemia in normal subjects, and pramlintide does not interfere with recovery from insulin-induced hypoglycaemia<sup>[227]</sup>.

**Efficacy:** There are several randomized controlled trials in T2DM that shows its efficacy; for example when added pramlintide to existing insulin therapy with or without a sulfonylurea or metformin, reductions in HbA1c (mean 0.62%) and weight (1.4 kg) were seen with 120 mcg but not 90 mcg of pramlintide given twice daily<sup>[228]</sup>. In a 24-wk trial or without oral agents had similar glycaemic efficacy as the addition of premeal rapid acting insulin analogs (HbA1c reduction of approximately 1%)<sup>[229]</sup>. Patients randomly assigned to pramlintide maintained their weight, whereas those assigned to rapid acting insulin gained weight (mean 4.7 kg). Pramlintide was associated with fewer hypoglycaemic events compared with prandial insulin. In addition to modest reductions in HbA1c and body weight, pramlintide has been associated with reductions in postprandial glucose excursions and in surrogate markers of cardiovascular risk and oxidative stress<sup>[230,231]</sup>.

**Side effects:** The most frequent side effect is nausea and generally dissipates by four weeks. Pramlintide should not be administered to patients with severe hypoglycaemia unawareness. Pramlintide should only be administered before meals that contain at least 250 calories or 30 g of carbohydrates. The recommended initial dose for T2DM is 60 mcg, titrated upward as tolerated to 120 mcg with each meal.

## TREATMENT OF T2DM IN OLDER PATIENTS

Elderly people with diabetes have a risk of developing macrovascular and microvascular complications, similar

to that of younger patients with diabetes. In addition, they have a higher rate of lower limb amputations, and other complications than any other age group<sup>[232,233]</sup>; and those  $\geq 75$  years have a higher rate of most complications than those between 65 and 74 years. Older people  $> 75$  years have a significant increase in death by hypoglycaemia, and visits to the emergency room for hypoglycaemia, compared to the general population with diabetes<sup>[234]</sup>.

Therefore, older people with diabetes have a number of characteristics that will influence their treatment, such as<sup>[235]</sup>: (1) presence of high co-morbidities; (2) presence of cognitive and functional impairment (falls); (3) polypharmacy; (4) visual and hearing impairment; (5) decreased physical activity; (6) high risk of hypoglycaemia; (7) common situations of social isolation and dependence. Depression; (8) nutrition-related problems; and (9) heterogeneity in terms of clinical presentation of the diabetes (diabetes duration, co-morbidities, functional status, life expectancy).

Based on all the above, the treatment of diabetes in the elderly people should achieve the following objectives: (1) to avoid disability, ensuring the best quality of life; (2) to avoid side effects of treatment, especially the most associated with impaired quality of life such as hypoglycaemia and falls; and (3) to have a global vision of the patient, introducing competitive risks in the decision-making process.

The initial treatment of T2DM in elderly patients is similar to that of younger patients, and includes changes in the lifestyle, with weight reduction, although most of elderly patients with T2DM will need drug treatment throughout his life.

### **Lifestyle modification**

Changes in lifestyle are very important in the treatment of diabetes at any age, but they deserve special considerations for the elderly. In the Diabetes Prevention Program, people  $< 60$  years of age improved their glycaemic control over time, due in part to better adapt to changes in lifestyle, compared with other younger age groups<sup>[236,237]</sup>.

**Nutritional needs:** Although calorie needs decrease with age, macronutrient needs will be similar throughout adulthood. Older people with diabetes are at risk of malnutrition from anorexia, altered taste and smell, difficulty swallowing, oral and dental problems, and functional alterations; major difficulties in the preparation and consumption of food. The Mini Nutritional Assessment, a questionnaire designed to detect malnutrition, is very easy to use and has proved useful in diabetes elderly patients<sup>[238]</sup>.

Nutritional recommendations should take into account the customs of the patients, their preferences and their personal goals and skills. When the regular intake does not meet the nutritional needs, a number of modifications, such as recommending fewer meals but more frequent, change the texture of foods, forti-

fying common foods, or add nutritional supplements between meals will be necessary. Overweight and obesity are common among the elderly. BMI is not useful in some older people due to changes in body composition with age<sup>[239]</sup>. Sarcopenia can occur in either overweight or underweight elderly. Moreover, obesity is often accompanied by decreased physical activity and increased frailty<sup>[240]</sup>. The unintentional weight loss in overweight or obese older people could worsen sarcopenia, bone mineral density and nutritional deficits<sup>[241,242]</sup>. Strategies that combine physical activity with nutritional therapy in older patients with diabetes, will lead to improved physical performance and a reduction of cardiometabolic risk<sup>[240,241]</sup>.

The caloric intake in the elderly should be between 25 and 35 kcal/kg per day<sup>[243]</sup>. Protein should provide 15%-20% of total calories, fat 30% maximum, avoiding saturated fats and trans fats, and promoting the consumption of monounsaturated fats and omega 3 fatty acids, and carbohydrates 50%-55% based on complex carbohydrates. A dietary fiber intake of about 14 g/1000 kcal is recommended, and they may also require calcium and vitamin D and vitamin B12 supplements. Fluid intake should be 30 mL/kg per day, with a minimum intake of 1500 mL/d, which may be increased in situations such as fever, infections, high temperatures, or excessive losses in urine and feces; or decreased in case of advanced renal insufficiency, or in states of fluid retention such as heart failure and liver cirrhosis<sup>[243]</sup>.

**Physical activity:** In older people with diabetes, muscle mass and strength decrease with age, worsening by complications of diabetes, co-morbidities and hospitalizations. People with diabetes of long duration and high levels of HbA1c, have less muscle strength per unit of muscle mass, that people without diabetes of similar age and BMI, and that people with diabetes of short duration and better glycaemic control<sup>[244]</sup>. Increased physical activity will improve the functional status of the elderly with or without diabetes<sup>[245]</sup>. In the elderly, mild physical activity is related with increased physical health and psychosocial well-being<sup>[246]</sup>, so that in these people with diabetes, healthy, it is recommended to perform the same exercise as other adults with diabetes<sup>[42]</sup>. Older patients with poorer health, will benefit even a modest increase in physical activity. Finally, patients at risk of falls should be referred to a physiotherapist for muscle and exercise balance.

### **Pharmacologic treatment**

Older patients have an increased risk of adverse events related to drugs due to pharmacokinetic changes as decreased renal elimination, and pharmacodynamics changes, age related, such as increased sensitivity to certain medications, which can affect at their disposal. These changes may result in an increased risk of hypoglycaemia, the need to reduce the dose of certain medicines and monitor renal function to minimize

adverse effects<sup>[247,248]</sup>. It is important to select drugs with a strong benefit/risk ratio, to provide efficacy, persistence and safety of treatment. Usually, in older people with diabetes is recommended to start treatment with antidiabetic at low doses, and titrate the dose progressively according to response, without reaching the maximum dose, due to the risk of increased side effects without increasing efficiency<sup>[249]</sup>.

Knowledge of the advantages and disadvantages of each family of antidiabetic drugs will help clinicians individualize treatment of elderly patients with T2DM<sup>[6]</sup>.

**Metformin:** Metformin remains the drug of choice for first-line treatment of T2DM in any age group, including the elderly. Its low risk of hypoglycaemia, its potential benefits in patients with stable cardiovascular disease<sup>[250]</sup> or heart failure, and its low cost, makes it a beneficial drug for older people. However its side effects such as gastrointestinal intolerance, vitamin B12 deficiency and weight loss, do not recommend its use in frailty patients. Although the risk of lactic acidosis is minimal, it is recommended to monitor renal function frequently, reduce the dose if the eGFR is between 30-60 mL/min<sup>[242]</sup>, and do not use it with eGFR < 30 mL/min<sup>[249-251]</sup>. Moreover, metformin should not be used in situations of tissue hypoxia, acute intercurrent disease, respiratory failure, acute heart failure, hepatic failure, administration of iodinated contrast, and risk of functional renal impairment (vomiting, diarrhoea). It is recommended to start with a low dose of 425 mg/d and titrate up to 1700 mg/d maximum, because with higher doses does not increase efficiency but increases side effects.

**Sulfonylureas:** They are also cheaper drugs, but due to its high risk of hypoglycaemia, should be utilized carefully in elderly. Hypoglycaemia appears more frequently with long-acting sulfonylureas such as chlorpropamide, glibenclamide and glimepiride, especially in older adults who develop severe and prolonged hypoglycaemia. We must stop using long-acting sulfonylureas in older adults<sup>[252]</sup>, being preferable the use of shorter-acting sulfonylureas such as glidazide and glipizide<sup>[253]</sup>. Circumstances that influence the occurrence of sulfonylureas-induced hypoglycaemia in the elderly are: (1) after exercise; (2) missed meals, eat poorly, without meal time, or abuse alcohol; (3) existence of impaired renal or cardiac function or intercurrent gastrointestinal disease; (4) after being in the hospital<sup>[254]</sup>; and (5) by associating salicylates, sulfonamides, fibric acid derivatives such as gemfibrozil, and warfarin<sup>[255]</sup>.

On the other hand, these drugs produce weight gain, and its use is limited in renal failure because of the high risk of hypoglycaemia. Furthermore, the large amount of drug interactions, interfere their use in the elderly.

**Meglitinides:** Repaglinide and nateglinide are designed

to control postprandial glycaemia, so that its duration is short and they require more frequent administrations with meals than sulfonylureas. Moreover, are more expensive, which limits its use in older people, especially in patients with polypharmacy. They lead a lower risk of hypoglycaemia than sulfonylureas<sup>[256]</sup>, especially in patients who do not a set meal schedule<sup>[72]</sup>, but they have a similar risk for weight gain. In addition, repaglinide, for its mainly biliary elimination, can be utilized in patients with moderate or advanced renal impairment<sup>[257]</sup>, and could be utilized as first-line in patients with impaired renal function when they are intolerant to metformin and sulfonylureas, or are contraindicated. It should not be associated with drugs that act by activating or inhibiting cytochrome P450, such as gemfibrozil, because of the high risk of hypoglycaemia.

**Alpha-glycosidase inhibitors:** Acarbose and miglitol are drugs that are intended to control postprandial blood glucose, with low risk of hypoglycemia, which are theoretically attractive to treat older people<sup>[258]</sup>. However gastrointestinal effects, low efficiency, more frequent daily doses, and cost limit their use. They can alter the levels of digoxin and acenocumarol.

**Thiazolidinediones:** Although TZD do not increase the risk of hypoglycaemia, and pioglitazone may be beneficial in patients in secondary prevention<sup>[259]</sup>, the high cost and side effects that induce as weight gain, macular oedema, fluid retention, increased risk of heart failure and bone fractures, and possible risk of bladder cancer<sup>[260]</sup>, limit their use in the elderly<sup>[261]</sup>.

**DPP-4 inhibitors:** iDPP4 inhibitors are once-a-day oral agents which can be used safely in elderly patients. They are very beneficial agents for the treatment of T2DM in the elderly since they control both basal and postprandial hyperglycaemia, with good tolerability, low risk of hypoglycaemia, and without significant drug interactions, or weight gain. These agents do not require dose adjustment in patients with advanced age. Although vildagliptin has demonstrated efficacy and safety in patients  $\geq 75$  years<sup>[262]</sup>, data safety in these patients is very limited. Linagliptin do not require dose adjustment in patients with renal impairment; vildagliptin at doses of 50 mg/d can be employed at any degree of renal failure; saxagliptin half-dose (2.5 mg/d) can be used in ESRD; and sitagliptin dose should be adjusted to the degree of renal insufficiency: 50 mg/d if the eGFR is between 30-50 mL/min, and 25 mg/d if < 30 mL/min. Finally, vildagliptin requires monitoring of liver function.

**iSGLT2:** The iSGLT2 dapagliflozin, canagliflozin and empagliflozin, represent a new class of oral hypoglycaemic agents that increase the urinary excretion of glucose. This effect results in lower blood glucose levels in an insulin-independent manner, with a lower risk of hypoglycaemia, as well as mild diuresis<sup>[263]</sup>. The



increase in glycosuria and diuresis produced, results in a reduction in weight and blood pressure. Because of these actions can be very attractive in the treatment of T2DM in the elderly<sup>[264,265]</sup>. However should not be utilized with an eGFR < 60 mL/min. Moreover, by inducing osmotic diuresis may increase the risk of dehydration, electrolyte abnormalities and weight loss that could limit its use in frail elderly patients. A common side effect of iSGLT2 is an increased incidence of genital and urinary infections, so they must be used with caution in elderly patients at increased risk of developing these infections or those with urinary incontinence<sup>[266]</sup>.

**RA-GLP1:** The RA-GLP1 exenatide, liraglutide, lixisenatide, albiglutide and dulaglutide, will control both basal and postprandial hyperglycaemia with a low risk of hypoglycaemia. The drug-related effects such as nausea, vomiting, decreased appetite and weight loss can be a problem for frail elderly patients; however may be an option in those not vulnerable, obese elderly patients with good performance status where weight loss is a priority<sup>[200]</sup>, as in those with knee osteoarthritis, sleep apnoea syndrome, hypoventilation, etc. Its use is not recommended in patients with an eGFR < 50 mL/min. There is little experience in patients  $\geq$  75 years, and its high cost and subcutaneous administration will limit its use in older patients.

**Insulin:** Insulin treatment can be utilized to achieve the goals of glycaemic control in selected older patients with T2DM, with similar efficacy and risk of hypoglycaemia than in younger patients. Before prescribing insulin in elderly subjects, we should think about the risk of hypoglycaemia related with this agent. The use of multiple daily injections of insulin, or by continuous subcutaneous insulin infusion in healthy elderly patients (mean age 66 years), has proven to be effective with a low rate of hypoglycaemia<sup>[267]</sup>. Also, the addition of long-acting insulin in elderly patients with T2DM (mean age 69 years) was as effective in achieving the HbA1c goals, without increased rate of hypoglycaemia, than in younger people (mean age 53 years)<sup>[268]</sup>. However there are few publications on the use of these insulin regimens in patients  $\geq$  75 years or in elderly patients with several co-morbidities, and/or a functional limitation. Visual or manual dexterity problems can be difficult to insulin therapy in some older patients. The use of insulin delivery devices will facilitate this work, selecting the one that best suits the skills and abilities of the patient. The risk of hypoglycaemia and weight gain will be lower with the use of insulin analogues compared to human insulins, and are preferred in elderly, despite their higher cost<sup>[208,269]</sup>, especially if there is a high risk of hypoglycaemia as in the frail or institutionalized elderly. Also, when necessary, the insulin analogs are preferable to short regular human insulin, due to its lower rate of hypoglycaemia<sup>[270]</sup>. Insulinization, especially in frail elderly, should start with a single daily dose of long-

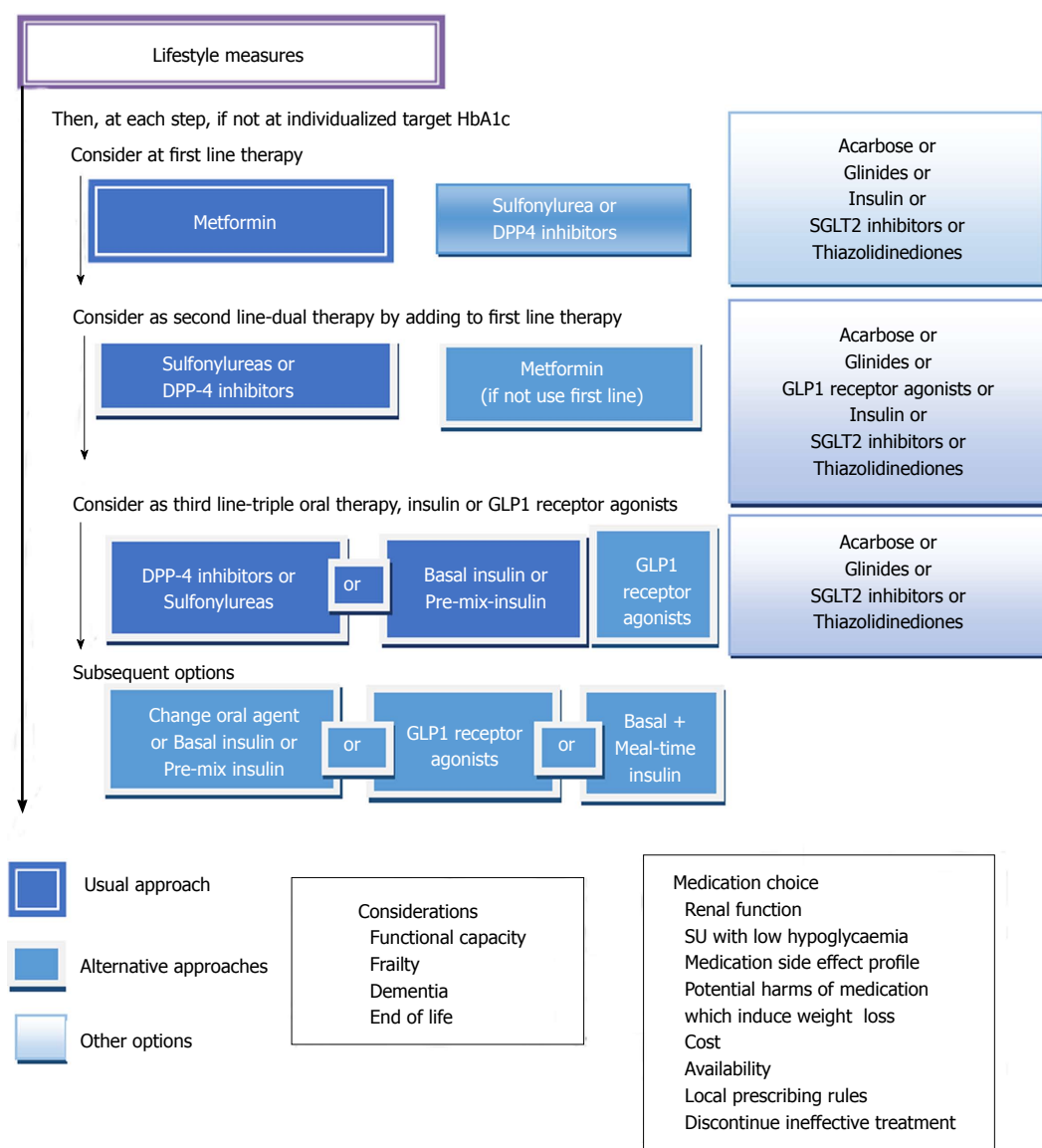
acting insulin (0.1-0.2 IU/kg), lower than in younger patients, to avoid hypoglycaemia. Figure 1 shows the International Diabetes Federation Global Guidelines for managing older people with T2DM<sup>[271]</sup>.

## TREATMENT OF T2DM IN PATIENTS WITH CKD

Before choosing a hypoglycaemic agent, we must consider the existence of an impairment renal function (Figure 2). Management of T2DM in patients with renal impairment is a complex process that requires a comprehensive approach. Clinicians must be aware that as renal function worsens, abnormalities in glucose homeostasis develop, affecting secretion, clearance, and peripheral tissue sensitivity to insulin<sup>[272]</sup>. CKD diagnosis adds risk factors for hypoglycaemia to those already present in patients with diabetes due to accumulation of uremic toxins, which lead to lower hepatic and renal insulin degradation, and also as a result of decreased renal gluconeogenesis, uremic malnutrition, and deficient catecholamine release<sup>[273]</sup>. Some of the additional factors are altered drug metabolism, drug-drug interactions, albuminuria, autonomic neuropathy, anorexia, malnutrition, infections, problems linked to dialysis, related cardiac and hepatic disease, and impaired renal glucose release<sup>[274,275]</sup>. On the other hand, both hypoglycaemia and CKD are related with increased morbidity and mortality from cardiovascular disease<sup>[276-278]</sup>. Many drugs are available for treatment of T2DM. Although all drugs can be utilized in patients with mild renal impairment<sup>[6,279]</sup>, therapeutic choices for patients with moderate to severe CKD and ESRD are reduced, since drug or metabolite accumulation may occur due to a reduced GFR resulting in increasing side effects. In this case, some drugs are not recommended, while others can be used with dose adjustment.

### Pharmacologic treatment

**Metformin:** The incidence of lactic acidosis in the setting of metformin therapy is low, and the drug is not necessarily responsible when lactic acidosis occurs in patients taking this medication<sup>[65]</sup>. Although drug levels are higher in those with kidney disease, levels are still maintained largely within the therapeutic range<sup>[280,281]</sup> and lactate levels are not substantially increased when metformin is utilized in those with reduced GFR<sup>[282-285]</sup>. The recommendations for use of metformin based on eGFR are shown in Figure 3<sup>[247]</sup>. However, the main problem for metformin treatment in CKD patients is the prevention of intoxication. Dosage guidelines for CKD patients have recently been published<sup>[286]</sup>. These recommend the following maximum daily doses related to creatinine clearance: 3 g (120 mL/min); 2 g (60 mL/min); 1 g (30 mL/min); 500 mg (15 mL/min). Moreover, Lipska *et al.*<sup>[247]</sup> have proposed a possible approach to metformin prescribing in the setting of CKD. The physician contemplating metformin treatment



**Figure 1 Global Guidelines for managing older people with type 2 diabetes.** International Diabetes Federation<sup>[272]</sup>. SGLT2: Sodium glucose co-transporter-2; GLP-1: Glucagon-like peptide-1; DPP-4: Dipeptidyl peptidase-4.

in a CKD patient should also address other problems. He should be advised to temporarily cease therapy if he develops sudden weight loss or acute illness, particularly if accompanied by vomiting and diarrhoea. X-ray contrast can occasionally cause acute renal insufficiency. In accordance with recent guidelines<sup>[287]</sup>, patients with an eGFR < 45 mL/min should stop metformin 48 h before contrast investigations, and restart 48 h after. Other contraindications, *e.g.*, liver disease and pregnancy, remain.

**Sulfonylureas:** Sulfonylureas can cause unregulated insulin release and lead to severe hypoglycaemia that can be particularly serious in the presence of CKD<sup>[288]</sup>, due to the accumulation of active metabolites. Long-acting sulfonylureas like glyburide and chlorpropamide are more notorious for causing hypoglycaemia<sup>[289]</sup>. Shorter-acting sulfonylureas as glimepiride, glipizide

and gliclazide agents are relatively safe and preferred in patients with CKD<sup>[290]</sup>. Major therapeutic considerations of sulfonylureas in patients with CKD and diabetes are<sup>[279,291-293]</sup>: (1) Glibenclamide should be prescribed with caution in patients with an eGFR 60-90 mL/min, and cannot be used in patients with an eGFR < 60 mL/min; (2) Glimepiride can be utilized in patients with an eGFR of < 60 mL/min, and dosage adjustment is required if the eGFR is < 30 mL/min. Begin at 1 mg daily or switch to another drug if the eGFR is < 15 mL/min; (3) Gliclazide is less than 1% excreted unchanged by the kidneys and does not have active metabolites<sup>[294]</sup>. It is recommended in subjects with an eGFR of 30-60 mL/min, need to reduce dose if the eGFR is < 30 mL/min, and it's not recommended if the eGFR is < 15 mL/min; and (4) Glipizide does not increase hypoglycaemia in patients with CKD. Can be utilized in all stages of CKD with caution and with dose reduction.

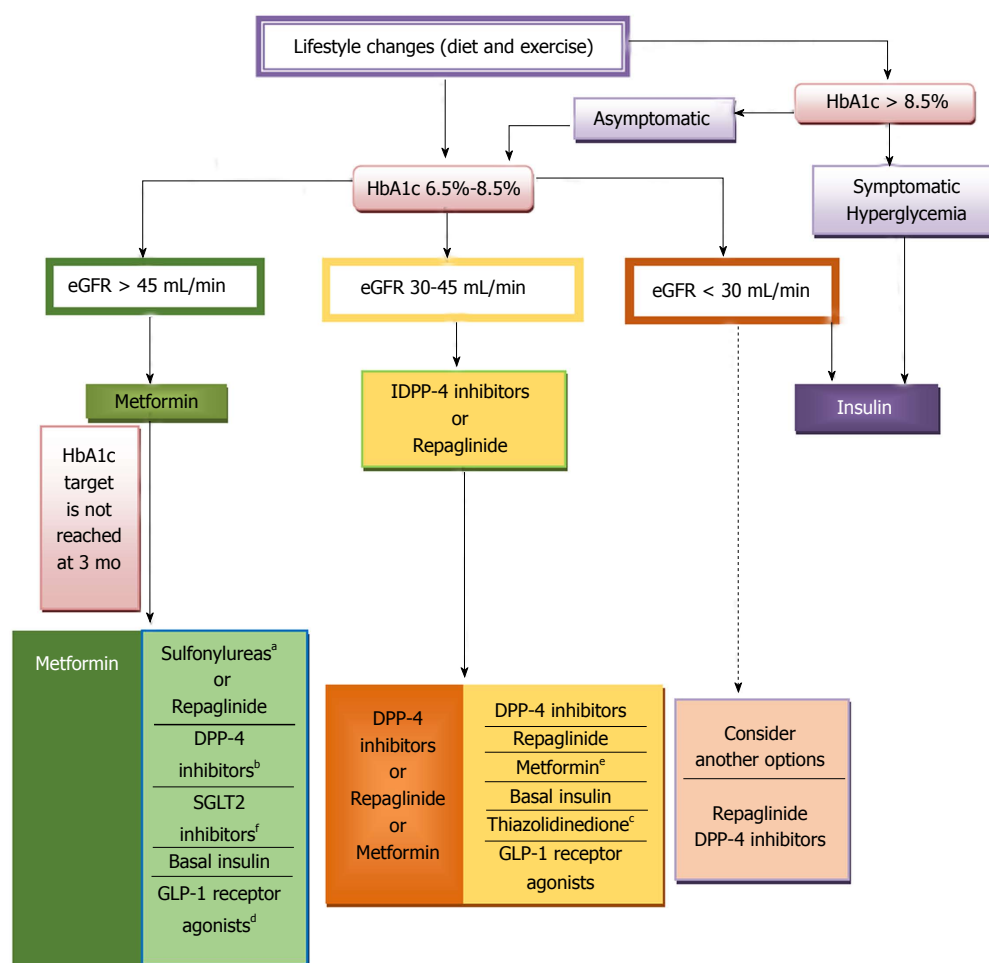
	eGFR > 60 mL/min	eGFR 45-60 mL/min	eGFR 30-45 mL/min	eGFR < 30 mL/min
Metformin	—————→			
Glibenclamide	————→			
Gliclazide	————→			
Glimepiride	————→			
Glipizide	————→			
Repaglinide	————→			
Nateglinide	————→			
Acarbose	————→			
Miglitol	————→			
Pioglitazone	————→			
Sitagliptin	————→			
Vildagliptin	————→			
Saxagliptin	————→			
Linagliptin	————→			
Alogliptin	————→			
Dapagliflozin	————→			
Canagliflozin	————→			
Empagliflozin	————→			
Exenatide	————→			
Liraglutide	————→			
Lixisenatide	————→			
Albiglutide	————→			
Dulaglutide	————→			
Insulin	————→			

————→ No dose adjustment is required      .....→ Dose adjustment is required

**Figure 2** Recommendations for use of antidiabetic agents based on estimated glomerular filtration rate. Adapted from Zanchi *et al*<sup>[292]</sup>. eGFR: Estimated glomerular filtration rate.

**Meglitinides:** Both repaglinide and nateglinide are primarily metabolized in the liver, and generally, dose adjustment is not required for either of these agents. Therefore, their risk of hypoglycaemia is lower, and they are more effective for postprandial glycaemic control. Thus, at first, they may be employed in patients with CKD, without dose adjustment<sup>[295]</sup>. Repaglinide is mostly metabolised by the liver and could therefore be utilized in patients with low renal function, although some dose adjustment is required<sup>[296]</sup>. Nateglinide is rapidly degraded by the liver to mostly inactive or weakly active metabolites which are eliminated in the urine<sup>[297]</sup>, also so can be considered patients with poor renal function, again with dose reduction. In conclusion, repaglinide and nateglinide can be prescribed in all stages of CKD with caution and dose reduction is necessary if the eGFR is < 30 mL/min<sup>[279,291,297,298]</sup>.

**Alpha-glucosidase inhibitors:** As only less than 2% of an oral dose of acarbose was absorbed as active drug, patients with an eGFR < 25 mL/min attained increases about fivefold higher for peak plasma concentration of acarbose and six fold higher for AUC values than subjects with normal renal function<sup>[95]</sup>. Miglitol is systematically absorbed but is not metabolized, and is rapidly eliminated by renal excretion as unchanged drug<sup>[299]</sup>. Patients with an eGFR < 25 mL/min taking miglitol 25 mg three times daily showed a twofold increase in miglitol plasma levels when compared with patients with an eGFR > 60 mL/min<sup>[300]</sup>. Voglibose, an alpha-glucosidase inhibitor only commercialized in Japan, has no renal excretion, and two studies showed that it can be safely utilized in diabetic patients on haemodialysis, in combination with pioglitazone or mitiglinide<sup>[300,301]</sup>. In conclusion, alpha-glucosidase



**Figure 3 Therapeutic algorithm in patients with type 2 diabetes mellitus and chronic kidney disease.** Adapted from Gómez-Huelgas *et al.*<sup>[338]</sup>. <sup>a</sup>Avoid Glibenclamide. Use Glizide, Glipizide and Gliquidone. Use Glimepiride only if eGFR > 60 mL/min; <sup>b</sup>Dose adjustment, except linagliptin; <sup>c</sup>Monitor fluid retention; <sup>d</sup>Adjust doses of exenatide and lixisenatide; <sup>e</sup>Generally not use Metformin. Use only half the dose and monitor renal function; <sup>f</sup>Not recommended if eGFR < 60 mL/min. eGFR: Estimated glomerular filtration rate; HbA1c: Glycated haemoglobin; GLP-1: Glucagon-like peptide-1; SGLT2: Sodium-glucose co-transporter 2; DPP-4: Dipeptidyl peptidase-4.

inhibitors acarbose and miglitol cannot be used if the eGFR is < 25 mL/min or the serum creatinine level is > 2 mg/dL<sup>[279,291,293,302]</sup>, while voglibose can be used in all stages of CKD including haemodialysis<sup>[300,301]</sup>.

**Thiazolidinediones:** Pioglitazone and rosiglitazone (only available in United States) are mainly metabolized in the liver and although a significant amount of active metabolites are eliminated in the urine; there is no need dose adjustment for either of these agents for patients with CKD<sup>[303]</sup>. However, both TZD cause fluid retention and increase the risk of heart failure, a problem that may be worse in patients with CKD. Although no dose adjustment in patients with CKD stages 3 to 5 is recommended<sup>[290]</sup>, its use in patients with CKD should be balanced with the possibility of worsening of fluid retention and fractures, the latter particularly in patients with underlying bone disease<sup>[290,304,305]</sup>.

**DPP-4 inhibitors:** iDPP4 are effective at lowering HbA1c in T2DM patients with moderate to severe renal impairment<sup>[304]</sup>. All iDPP4 differ in their renal

excretion and therefore should be handled differently in patients with impairment renal function. Results from dedicated pharmacokinetics studies in subjects with various degrees of renal impairment suggest that the daily doses of all iDPP4 except linagliptin should be adjusted according to eGFR<sup>[305]</sup>. Several studies have demonstrated that the glucose-lowering efficacy is maintained while a good safety profile when reduced doses of these gliptins are utilized in patients with renal impairment<sup>[306-309]</sup>. On the other hand, linagliptin not require any dose adjustment in case of renal impairment, because is mainly excreted by the biliary route<sup>[310]</sup>, and can be used in patients with all degrees of CKD<sup>[311]</sup>. Sitagliptin is largely excreted unchanged in the urine (87%) or *via* the feces (13%). No dose adjustment is necessary in patients with an eGFR > 50 mL/min, and can be utilized with dose reduction in patients with moderate to severe renal impairment<sup>[279,291,292,312]</sup>. The dose should be reduced by half in patients with an eGFR 30-50 mL/min, and a quarter in those with an eGFR < 30 mL/min or requiring dialysis. Around 80% of vildagliptin dose is metabolised



mostly in the kidneys into non-active metabolites which are then renally excreted (85%) or recovered in the feces (15%)<sup>[313]</sup>. Vildagliptin not need dose adjustment in patients whose eGFR is > 50 mL/min and with caution in those with ESRD. The dose should be reduced by half in patients with moderate to severe renal impairment<sup>[279,291,292]</sup>. Saxagliptin is metabolised mainly in the liver to an active metabolite that is renally excreted, with approximately 20% of a dose being recovered unchanged in the urine and 20%-50% as metabolites<sup>[314]</sup>. No dose adjustment is required for patients with an eGFR > 50 mL/min, whereas the dose should be reduced by half in patients with moderate or severe renal impairment<sup>[279,291,292]</sup>. Vildagliptin can not be utilized in those on renal replacement therapy. Linagliptin is excreted almost entirely unchanged in bile, and its elimination is essentially *via* the feces<sup>[315]</sup>. No dose adjustment is required in patients with any stage of CKD<sup>[279,291,292]</sup> including, with caution, those requiring renal replacement therapy<sup>[316,317]</sup>. Alogliptin does not suffer appreciable metabolism and around 80% is eliminated unchanged in urine<sup>[318]</sup>. No dose adjustment is required for patients with an eGFR > 50 mL/min. Alogliptin dose adjustments are recommended for patients with moderate to severe renal impairment, including those with ESRD requiring dialysis. The dose should be reduced by half in patients with an eGFR 30-50 mL/min, and a quarter in patients with an eGFR < 30 mL/min or ESRD<sup>[279]</sup>.

**Sodium-glucose co-transporter 2 inhibitors:** iSGLT2 decrease plasma glucose concentration by inhibiting the reabsorption of glucose by the kidney, which in turn, is a function of plasma glucose concentration and GFR. Because these agents rely on GFR to increase urinary glucose excretion, they are expected to have a decreased effect as kidney function declines. Studies examining the efficacy of iSGLT2 inhibition in patients with diabetes have been reported for a number of iSGLT2 including canagliflozin<sup>[319]</sup>, dapagliflozin<sup>[320]</sup>, empagliflozin<sup>[321]</sup> and ipragliflozin<sup>[322]</sup>. As expected, the efficacy of iSGLT2 decreases as kidneys function decreases<sup>[320-322]</sup>. Although renal function does not seem to be affected<sup>[141]</sup>, its use in patients with moderate to severe CKD is not recommended. Dapagliflozin is not recommended if the eGFR is < 60 mL/min. In patients with an eGFR < 60 mL/min., canagliflozin and empagliflozin should not be initiated, but they may be continued in patients already taking the medications. Patients with an eGFR of 45-60 mL/min should be of the lower doses once a day, and both medications are contraindicated in patients with an eGFR < 45 mL/min, or on dialysis<sup>[139]</sup>.

**RA-GLP1:** Due to the effect of these agents on gastric emptying, side effects are mainly gastrointestinal: Nausea, vomiting and diarrhoea. These gastrointestinal side effects with recurrent vomiting will lead to dehydration and secondary acute renal failure<sup>[323]</sup>. Exenatide

is extensively renally eliminated by glomerular filtration and undergoes degradation by the kidneys to small, inactive peptide fragments<sup>[324]</sup>. There is reduced clearance in people with renal impairment<sup>[325]</sup>. Exenatide can be utilized in patients with an eGFR > 50 mL/min, whereas cannot be used in patients with an eGFR < 30 mL/min. In subjects with an eGFR of 30-60 mL/min, exenatide should only be employed with great caution and a lower doses<sup>[279,291,302]</sup>. Liraglutide is metabolised in a similar manner to large proteins, and its shows no reduced clearance in patients with renal impairment, and undergoes only minimal renal excretion<sup>[326]</sup>. No dose adjustment is required in subjects with an eGFR > 30 mL/min. Limited data are available in patients with an eGFR < 30 mL/min and ESRD, and should not be used in these populations<sup>[326]</sup>. As a peptide, lixisenatide is eliminated through glomerular filtration, followed by tubular reabsorption and subsequent metabolic degradation. No dose adjustment is recommended for patients with an eGFR > 50 mL/min, but as there is limited therapeutic experience in patients with an eGFR 30-50 mL/min, lixisenatide should be utilized with caution and is contraindicated in those with an eGFR < 30 mL/min and with ESRD<sup>[327]</sup>. Albiglutide is a protein, so the expected metabolic pathway is degradation to small peptides and amino acids by ubiquitous proteolytic enzymes. No dose adjustment is necessary in subjects with an eGFR > 30 mL/min. Limited data are available in subjects with an eGFR < 30 mL/min and should be used with caution in these populations<sup>[328]</sup>. Finally, dulaglutide is presumed to be degraded into its component amino acids by general protein pathways. No dose adjustment is recommended in subjects with renal impairment including ESRD. Limited data are available in patients with an eGFR < 30 mL/min, and should be employed with caution<sup>[329]</sup>.

**Insulin:** Insulin is generally considered to be safe in patients with a reduced kidney function. Because of their low levels of degradation, insulin prolongs its half life when there is an impairment in kidney function<sup>[330]</sup>. As a result the risk of hypoglycaemic events is 5 times higher than in subjects without impairment renal function<sup>[331]</sup>. Almost 50% of circulating insulin is cleared by the kidney *via* glomerular filtration and subsequent luminal reabsorption of insulin by proximal tubular cells by means of endocytosis, or *via* diffusion of insulin from peritubular capillaries and subsequent binding of insulin to the contraluminal membranes of tubular cells. In insulin-treated T2DM patients, insulin doses should be reduced by 25% when the eGFR is between 10-50 mL/min, and by 50% when the eGFR is < 10 mL/min<sup>[332,333]</sup>. As for human insulin, the pharmacokinetic/pharmacodynamic profiles for insulin analogs may be influenced by many variables including renal function, although the available data are rather scarce<sup>[334]</sup>. Reduction of initial glargine/gulisine insulin weight-based dosing in hospitalized patients with T2DM and renal impairment reduced the frequency

of hypoglycaemia by 50% without compromising the control of hyperglycaemia<sup>[335]</sup>. Short-acting insulin analog can also be utilized in haemodialysis patients with T2DM<sup>[336]</sup>.

Figure 3 shows the therapeutic algorithm for the treatment of patients with T2DM and CKD, proposed by the Spanish Working Group, sponsored by several scientific societies<sup>[337]</sup>.

## BARIATRIC SURGERY

Bariatric surgery could be an alternative in the treatment of obesity. Candidates for bariatric surgery are patients with a morbid obesity or those with a BMI > 35 kg/m<sup>2</sup> who also have co-morbidities, such as hypertension, T2DM or obstructive sleep apnoea. After this surgery, it was observed a metabolic response leading to decrease blood glucose with improvements or remission of diabetes. Moreover, bariatric surgery also improves the metabolic status, improving lipid profile and hypertension, thus decreasing cardiovascular risk<sup>[338]</sup>.

The improvement in glycaemic control, has been observed before the achievement of clinically significant weight loss. Although there are no consistent theories to explain the early improvement in T2DM after surgery, it seems a direct consequence of gastrointestinal anatomy restructuring that produces hormonal change and decreases food intake with an acute negative calorie balance<sup>[339]</sup>. This supports the idea that "metabolic surgery" is a definition more appropriate, and it refers a bariatric surgery in patients with less grade obesity than those who are traditionally eligible for bariatric surgery<sup>[338]</sup>. Despite this, it is necessary more investigation for known entirely the relationship between metabolic effects of bariatric surgery in overweight and in patients with obesity class I.

## THE FUTURE IN THE TREATMENT OF DIABETES

Unfortunately, all anti-diabetic agents have adverse effects, and are expensive. Therefore, the investigation of novel antidiabetic regimens, with less adverse effects and cheaper, is a major challenge for researchers.

### Polyphenols

Natural products containing high polyphenol levels as blackberries, red grapes, apricots, eggplant, coffee, cocoa and green tea can regulate glucose metabolism through different paths, such as restoring beta-cell integrity, enhancing insulin releasing activity, and increasing cellular glucose uptake, which can improve insulin resistance<sup>[340]</sup>.

### Smart insulin patch

A new smart insulin patch has been created. It is a thin square covered with more than 100 tiny needles. The

patch made of biocompatible materials works fast and it's easy to use. The patch consists of small painless needles that are packed together with insulin and glucose-sensitive enzymes in microscopic storage units. The patch releases these enzymes when blood glucose increases. In a mouse model, patch administration showed reduced glucose levels up to 9 h<sup>[341]</sup>. It is suggested that the patch could have a longer effect in diabetic humans since humans are more sensitive to insulin than mice.

### Dual-acting peptide

GLP1 and GIP are the two main incretin hormones that are released from the intestine in response to food intake. Both hormones stimulate glucose-dependent insulin secretion. Evidence from animal studies suggests that anti-obesity efficacy of GLP1 can be enhanced by co-administration with the incretin hormone GIP. Finan *et al.*<sup>[342]</sup> showed that an acylated version of GLP1 and GIP dual agonist, reduced weight (-18.8% vs -8.8%,  $P < 0.001$ ), food intake ( $P < 0.05$ ), fat mass ( $P < 0.001$ ) and blood glucose ( $P < 0.05$ ), compared to liraglutide. Also showed increases in plasma insulin and C-peptide more pronounced than liraglutide ( $P < 0.001$  for both). No differences in improved glycaemic control between these co-agonists and liraglutide were found. In T2DM patients they found a dose-dependent reductions of HbA1c, being -0.53% in patients treated with 4 mg of the dual agonist, and -1.11% in those treated with 30 mg, compared with placebo (-0.16%). The pharmacokinetics and pharmacodynamics results of co-activation of GLP1 and GIP receptors<sup>[343]</sup> are considered as a promising new strategy for the treatment of obese T2DM patients, to prolong the activity of GLP1 and GIP dual agonists, and for the future development of a possible once-weekly GLP-1 and GIP dual agonists drug candidate for the treatment of T2DM.

**GLP1 and Glucagon receptor dual agonism:** Glucagon and GLP1 have distinct receptors that are also structurally related<sup>[344]</sup>. Glucagon stimulates gluconeogenesis and glycogenolysis in the liver, raising blood glucose levels; while GLP1 reduce blood glucose levels by increasing insulin synthesis and secretion in the pancreas<sup>[345]</sup>. Administration of oxyntomodulin, a GLP1 receptor/glucagon receptor dual agonist peptide, to rodents<sup>[346-348]</sup> and humans<sup>[349,350]</sup>, resulted in a improvement of glucose metabolism by decreasing food intake and body weight, and increasing energy expenditure, more pronounced than those reported by GLP1. Moreover, weekly administration of PEGylated peptides reduced adiposity and improved glucose tolerance in diet-induced obese mice<sup>[351]</sup>, and sustained GLP1/glucagon dual agonism reverses obesity in diet-induce obese mice<sup>[352]</sup>. These co-agonist compounds also normalized glucagon, glucose and lipid metabolism and reduced liver steatosis, and is a novel therapeutic approach to the treatment of obesity in patients with

T2DM.

**GLP1 receptor agonist and Glucagon receptor antagonism activity:** GLP1/Glucagon hybrid peptides, a dual acting peptide that bind both receptors, for diabetes (DAPD) have been reported previously<sup>[344]</sup>, and more recently have been identified *in vitro*<sup>[353]</sup>. Administration of PEGylated DAPD in mice, showed a decrease in blood glucose by increasing insulin secretion GLP1-induced, and a rise in fasting glucagon levels following a glucagon challenge<sup>[354]</sup>. Moreover, unlike RA-GLP1, does not inhibit gastrointestinal motility and has not adverse events at this level.

#### **Basal insulin analogs with glucagon-like peptide-1 mimetics**

The combination of GLP1 mimetics with basal insulin reduced the risk of hypoglycaemia and weight gain induced for intensive insulin regimens in T2DM patients. Preliminary evidence suggests that the addition of a basal insulin to a GLP1 mimetic with or without oral therapy, provide improvements in basal and postprandial glucose control, with less weight gain, reduced risk of hypoglycaemia and increased satisfaction<sup>[188-190,355-358]</sup>. Data from the DUAL I extension (insulin-naïve patients not controlled with oral hypoglycaemic agents) and DUAL II (patients not controlled on basal insulin plus oral hypoglycaemic agents) randomized trials, the novel fixed combination of insulin degludec and liraglutide (IDegLira), effectively lowered HbA1c across a range of measures, implying suitability for patients with either early or advanced T2DM<sup>[359]</sup>. LixiLan is a new once-daily single injection fixed-ratio combination of lixisenatide, and insulin glargine. Results from the LixiLan-L trial, showed that LixiLan successfully met the primary study endpoint of demonstrating a statistically superior reduction in HbA1c compared with insulin glargine<sup>[360]</sup>.

#### **G protein-coupled receptor 119**

G protein-coupled receptor 119 (GPR119) agonists is a G protein-coupled receptor that is expressed predominantly in the pancreas and gastrointestinal tract in rodents and humans, as well as in the brain in rodents<sup>[361]</sup>. Activation of the receptor showed a reduction in food intake and body weight gain in rats<sup>[361]</sup>. GPR119 has also been shown to regulate incretin and insulin secretion<sup>[362-364]</sup>. New agents acting on this receptor have been suggested as novel treatments for obesity and diabetes<sup>[361,363,365]</sup>.

It is worth pointing out the potential advantages that could be obtained by the co-administration of a GPR119 agonist and a iDPP4. The role of these additional hormonal agents will required to clarify in the further study<sup>[366]</sup>.

#### **Oral RA-GLP1**

Currently, RA-GLP1s are available only as injectables,

either once daily or once weekly. Semaglutide is a long-acting RA-GLP1 that is also being developed as a once-weekly injectable. An oral semaglutide version leading to higher solubility and protection from enzymatic degradation is also being developed.

The phase 2 study<sup>[367]</sup> enrolled 632 adults with T2DM of 6 to 7 years duration, managed with lifestyle with or without metformin, and HbA1c 7.0% to 9.5% (mean, 7.9%). They were randomized to oral semaglutide in doses of 2.5, 5, 10, 20 or 40 mg once daily, or to placebo, or to open-label injected once-weekly 1.0-mg semaglutide. Patients started at 2.5 or 5 mg once daily and the higher-dose groups were titrated up at 4-wk intervals. The primary endpoint was change in HbA1c from baseline to week 26.

At 26 wk, mean HbA1c decreased dose-dependently with oral semaglutide, with drops ranging from 0.7% with 2.5 mg to 1.9% with 40 mg. Subcutaneous once-weekly semaglutide also produced a 1.9% drop in HbA1c, while the placebo group experienced a decrease of only 0.3% ( $P = 0.07$  for 2.5 mg vs placebo,  $P < 0.0001$  for other doses). For all the groups taking 5-mg oral semaglutide or higher doses, more than 80% of the patients achieved HbA1c values less than 7%, and the groups treated with 10-mg dose or more achieved mean HbA1c less than 6.5%. Fasting plasma glucose also dropped significantly, from a baseline of 170 mg/dL, with reductions ranging from 17 mg/dL with 2.5 mg to 51 mg/dL for the other oral doses ( $P = 0.08$  for 2.5 mg,  $P < 0.0001$  for other doses) and a reduction of 56 mg/dL with 1.0-mg subcutaneous semaglutide vs 1 mg/dL with placebo.

The proportion of patients achieving 5% or more weight loss was 21% to 71% in the oral group and 66% in subcutaneous group, compared with 13% in the placebo group.

None of the adverse events were considered serious and all were reported as mild to moderate in severity. Increases in lipase levels were greater in the oral and subcutaneous semaglutide groups, compared with placebo.

Based on these data, oral semaglutide is now being studied in a large phase 3 trial<sup>[368]</sup>.

#### **Oral insulin**

Oral administration of insulin is a novel treatment to improve glycaemic control in patients with T2DM. Oral insulin has a more physiological action than parenteral insulin. Due to its first pass through the liver, it reduces glycogenolysis, hepatic glucose production, and the risk of hypoglycaemia, compared with parenteral insulin. Currently, the data available in human trials suggest that could be a novel approach to the treatment of diabetes<sup>[369,370]</sup>.

There are several oral insulins in development: Short-acting insulins as ORMD-0801 (Oramed) and Capsulin (Diabetology) in phase 2 studies, and the IN-105 (Biocon) in phase 3 studies; and basal insulins,

such as the OI287GT (NN1956) (NovoNordisk).

### Dual inhibition of SGLT1 and SGLT2

Sotagliflozin is a dual inhibitor of SGLT1 and SGLT2 with approximately 20-fold selectivity for SGLT2 over SGLT1<sup>[371]</sup>. Animal pharmacology studies showed that sotagliflozin produced increased urinary glucose excretion, delivery of glucose to the caecum, increased postprandial GLP1 and peptide YY release, that were related with significant reductions in postprandial glucose<sup>[372,373]</sup>. Sotagliflozin was evaluated in patients with T2DM not controlled with metformin<sup>[372]</sup>. Sotagliflozin reduced fasting plasma glucose and HbA1c with a modest urinary glucose excretion, compared with selective iSGLT2. The high glycaemic efficacy observed with only modest urinary glucose excretion suggested that clinically relevant gastrointestinal SGLT1 inhibition was present. Phase 1 and phase 2 studies have identified special opportunities for synergy with iDPP-4 for treatment of patients with T2DM and renal impairment.

### Other treatments

Technosphere insulin, a new inhaled insulin represent an alternative to bolus insulin injections, but can be used concomitantly with basal insulin injections. Its hypoglycaemic effect is less than the rapid-acting insulin, but has less hypoglycaemias<sup>[374]</sup>. Major adverse effects are respiratory, with cough being the most prominent, and there is a small decrease in the forced expiratory volume in 1 s (FEV1) with technosphere insulin, consistent, no progressive, and reversible; so that patients must receive pulmonary function test periodically throughout therapy. Should be utilized with caution in patients who smoke and is contraindicated in patients with chronic lung disease.

New chitosan formulations of xanthine derivatives (CS-6, CS-7) have been synthesized as antidiabetic and antioxidant treatments. Formulations of chitosan 6 (CS-6) have shown to reduce blood glucose levels by 59.3%, with a recorded 4.53% HbA1c level<sup>[375]</sup>. These effects were more intense than the induced by pioglitazone (114.5 mg/dL vs 148.5 mg/dL), when used as standard antidiabetic medication. These results have shown the potential application of chitosan formulations of Xanthine 6 derivatives (CS-6) in the treatment of diabetes mellitus.

Recent studies have shown the dynamic role of zinc, an insulin mimetic, as a "cellular second messenger" in glucose homeostasis and in the control of insulin signaling<sup>[376]</sup>. Synthesis, secretion and insulin action are dependent on zinc and transporters. This suggests that zinc plays a role, previously not identified, where changes in the state of zinc over time can affect the activity of insulin. This is a novel area of investigation, and introduces a new class of useful drugs for diabetes pharmacotherapy.

Imeglimin is the first of the family of agents called "glimins" and, more specifically, is a tetrahydrotriazene

compound<sup>[377]</sup>. Laboratory studies<sup>[377,378]</sup> have shown that acts on impaired glucose uptake by muscle tissue, excessive hepatic gluconeogenesis, and increased apoptosis of beta cells. Imeglimin is still in development and human studies are limited. The few human studies recently published<sup>[377,379-381]</sup> show that reduces HbA1c and fasting glucose similar to sitagliptin and metformin, with a low incidence of side effects, especially hypoglycaemia. Currently, there is an ongoing trial that evaluated the safety and efficacy of imeglimin with insulin therapy or compared directly with insulin in patients newly diagnosed or treated with oral monotherapy, whose results have not yet been published<sup>[382]</sup>. Imeglimin seems to be a promising antidiabetic agent as monotherapy in the treatment of T2DM.

Recent studies reported a possible role of the G protein coupled receptor 40 (GPR40), also known as FFAR 1, in the regulation of beta-cell function<sup>[383]</sup>. It was reported that chronic treatment of male Zucker diabetic fatty (ZDF) rats (insulin resistant model with elevated blood glucose and FFAs levels) with CNX-011-67 (GPR40 agonist) increased insulin secretion, decreased blood glucose and reduced beta-cell apoptosis without affecting body weight<sup>[384]</sup>. From this study data it appears that CNX-011-67 could have the potential to provide good and durable glycaemic control in T2DM patients. Another study provided evidence that activation of GPR40 with CNX-011-67 stimulates glucose metabolism, improve glucose responsiveness and enhances insulin secretion, with the hope that pharmacological activation of GPR40 will prove beneficial for the treatment of T2DM<sup>[384]</sup>. TAK-875, a novel highly selective, orally bioavailable GPR40 agonist, significantly improved glycaemic control in patients with T2DM with a minimum risk of hypoglycaemia. The outcomes show that activation of FFAR1 is a viable therapeutic target for the treatment of T2DM<sup>[385]</sup>. According to current data it can be appreciated that beta-cell failure could be delayed or prevented by attaining and maintaining good glycaemic control. It is theoretically possible to inhibit multiple mechanisms by blocking the pathways leading to beta-cell apoptosis, and this is a challenge for the future.

Finally, *in vivo* studies, administration of hot water extracts of *Salacia chinensis* to diet-fed KK-A<sup>y</sup> mice, resulted in a significant reduction in the basal and postprandial blood glucose and HbA1c levels; with an improvement of glucose tolerance<sup>[386]</sup>. The active components, salacinol, kotalanol, and neokotalanol inhibited human  $\alpha$ -glucosidases as potently as they inhibited rat small intestinal  $\alpha$ -glucosidase. The results suggest that these sulfoniums can be good candidates as new type of anti-diabetic agents.

## CONCLUSION

While lifestyle modifications and metformin are the cornerstone of the initial management of T2DM, there is an increasing array of second and third-line pharma-



cological agents for this condition. At present there are different families of oral and injectable drugs, available for the treatment of T2DM. These include sulfonylureas, meglitinides, insulin, TZD and alpha-glucosidase inhibitors, and recently with the addition of RA-GLP1 receptor agonists, iDPP4 and iSGLT2. Moreover, insulin analogues that better simulate endogenous insulin secretion have been developed. Metformin remains the first choice of treatment for most patients. Other alternative or second-line treatment options should be individualized taking into consideration patient characteristics as degree of hyperglycaemia, presence of co-morbidities, and patient preference and ability to access treatments; and properties of the treatment such effectiveness and durability of lowering blood glucose, risk of hypoglycaemia, effectiveness in reducing diabetes complications, effect on body weight, side effects and contraindications. Although it does not appear that in the near future cure diabetes, novel safety and effective agents that will improve the quality of life of T2DM patients, are developing.

## REFERENCES

- International Diabetes Foundation.** Diabetes: facts and figures. [accessed 2016 Mar 22]. Available from: URL: <http://www.idf.org/WDD15-guide/facts-and-figures.html>
- DeFronzo RA, Bonadonna RC, Ferrannini E.** Pathogenesis of NIDDM. A balanced overview. *Diabetes Care* 1992; **15**: 318-368 [PMID: 1532777 DOI: 10.2337/diacare.15.3.318]
- Mazzone T, Chait A, Plutzky J.** Cardiovascular disease risk in type 2 diabetes mellitus: insights from mechanistic studies. *Lancet* 2008; **371**: 1800-1809 [PMID: 18502305 DOI: 10.1016/S0140-6736(08)60768-0]
- Ritz E, Rychlik I, Locatelli F, Halimi S.** End-stage renal failure in type 2 diabetes: A medical catastrophe of worldwide dimensions. *Am J Kidney Dis* 1999; **34**: 795-808 [PMID: 10561134 DOI: 10.1016/S0272-6386(99)70035-1]
- del Cañizo-Gómez FJ, Moreira-Andrés MN.** Cardiovascular risk factors in patients with type 2 diabetes. Do we follow the guidelines? *Diabetes Res Clin Pract* 2004; **65**: 125-133 [PMID: 15223224 DOI: 10.1016/j.diabres.2003.12.002]
- Inzucchi SE, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, Peters AL, Tsapas A, Wender R, Matthews DR.** Management of hyperglycemia in type 2 diabetes, 2015: a patient-centered approach: update to a position statement of the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care* 2015; **38**: 140-149 [PMID: 25538310 DOI: 10.2337/dc14-2441]
- Bagnasco A, Di Giacomo P, Da Rin Della Mora R, Catania G, Turci C, Rocco G, Sasso L.** Factors influencing self-management in patients with type 2 diabetes: a quantitative systematic review protocol. *J Adv Nurs* 2014; **70**: 187-200 [PMID: 23763567 DOI: 10.1111/jan.12178]
- Shen H, Edwards H, Courtney M, McDowell J, Wei J.** Barriers and facilitators to diabetes self-management: perspectives of older community dwellers and health professionals in China. *Int J Nurs Pract* 2013; **19**: 627-635 [PMID: 24330214 DOI: 10.1111/ijn.12114]
- Garber AJ, Abrahamson MJ, Barzilay JI, Blonde L, Bloomgarden ZT, Bush MA, Dagogo-Jack S, DeFronzo RA, Einhorn D, Fonseca VA, Garber JR, Garvey WT, Grunberger G, Handelsman Y, Henry RR, Hirsch IB, Jellinger PS, McGill JB, Mechanick JI, Rosenblit PD, Umpierrez GE.** Consensus statement by the american association of clinical endocrinologists and american college of endocrinology on the comprehensive type 2 diabetes management algorithm--2016 executive summary. *Endocr Pract* 2016; **22**: 84-113 [PMID: 26731084 DOI: 10.4158/EP151126.CS]
- National Diabetes Education Program (NDEP).** Guiding principles for the care of people with or at risk for diabetes. [accessed 2016 Jan]. Available from: URL: <http://www.niddk.nih.gov>
- Cappuccio FP, Cooper D, D'Elia L, Strazzullo P, Miller MA.** Sleep duration predicts cardiovascular outcomes: a systematic review and meta-analysis of prospective studies. *Eur Heart J* 2011; **32**: 1484-1492 [PMID: 21300732 DOI: 10.1093/eurheartj/ehr007]
- McNeil J, Doucet É, Chaput JP.** Inadequate sleep as a contributor to obesity and type 2 diabetes. *Can J Diabetes* 2013; **37**: 103-108 [PMID: 24070800 DOI: 10.1016/j.cjcd.2013.02.060]
- Nathan DM, Buse JB, Davidson MB, Ferrannini E, Holman RR, Sherwin R, Zinman B.** Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement of the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care* 2009; **32**: 193-203 [PMID: 18945920 DOI: 10.2337/dc08-9025]
- Esposito K, Maiorino MI, Ciotola M, Di Palo C, Scognamiglio P, Gicchino M, Petrizzo M, Saccomanno F, Beneduce F, Ceriello A, Giugliano D.** Effects of a Mediterranean-style diet on the need for antihyperglycemic drug therapy in patients with newly diagnosed type 2 diabetes: a randomized trial. *Ann Intern Med* 2009; **151**: 306-314 [PMID: 19721018 DOI: 10.7326/0003-4819-151-5-200909010-00004]
- Faulconbridge LF, Wadden TA, Rubin RR, Wing RR, Walkup MP, Fabricatore AN, Coday M, Van Dorsten B, Mount DL, Ewing LJ.** One-year changes in symptoms of depression and weight in overweight/obese individuals with type 2 diabetes in the Look AHEAD study. *Obesity* (Silver Spring) 2012; **20**: 783-793 [PMID: 22016099 DOI: 10.1038/oby.2011.315]
- Foster GD, Borradaile KE, Sanders MH, Millman R, Zammit G, Newman AB, Wadden TA, Kelley D, Wing RR, Pi-Sunyer FX, Reboussin D, Kuna ST.** A randomized study on the effect of weight loss on obstructive sleep apnea among obese patients with type 2 diabetes: the Sleep AHEAD study. *Arch Intern Med* 2009; **169**: 1619-1626 [PMID: 19786682]
- Phelan S, Kanaya AM, Subak LL, Hogan PE, Espeland MA, Wing RR, Burgio KL, DiLillo V, Gorin AA, West DS, Brown JS.** Weight loss prevents urinary incontinence in women with type 2 diabetes: results from the Look AHEAD trial. *J Urol* 2012; **187**: 939-944 [PMID: 22264468 DOI: 10.1016/j.juro.2011.10.139]
- Williamson DA, Rejeski J, Lang W, Van Dorsten B, Fabricatore AN, Toledo K.** Impact of a weight management program on health-related quality of life in overweight adults with type 2 diabetes. *Arch Intern Med* 2009; **169**: 163-171 [PMID: 19171813 DOI: 10.1001/archinternmed.2008.544]
- Franz MJ, Boucher JL, Green-Pastors J, Powers MA.** Evidence-based nutrition practice guidelines for diabetes and scope and standards of practice. *J Am Diet Assoc* 2008; **108**: S52-S58 [PMID: 18358257 DOI: 10.1016/j.jada.2008.01.021]
- Andrews RC, Cooper AR, Montgomery AA, Norcross AJ, Peters TJ, Sharp DJ, Jackson N, Fitzsimons K, Bright J, Coulman K, England CY, Gorton J, McLenaghan A, Paxton E, Polet A, Thompson C, Dayan CM.** Diet or diet plus physical activity versus usual care in patients with newly diagnosed type 2 diabetes: the Early ACTID randomised controlled trial. *Lancet* 2011; **378**: 129-139 [PMID: 21705068 DOI: 10.1016/S0140-6736(11)60442-X]
- Nguyen NT, Nguyen XM, Lane J, Wang P.** Relationship between obesity and diabetes in a US adult population: findings from the National Health and Nutrition Examination Survey, 1999-2006. *Obes Surg* 2011; **21**: 351-355 [PMID: 21128002 DOI: 10.1007/s11695-010-0335-4]
- Shoelson SE, Lee J, Goldfine AB.** Inflammation and insulin resistance. *J Clin Invest* 2006; **116**: 1793-1801 [PMID: 16823477 DOI: 10.1172/JCI29069]
- Gargallo Fernández Manuel M, Breton Lesmes I, Basulto**

- Marset J, Quiles Izquierdo J, Formiguera Sala X, Salas-Salvadó J. Evidence-based nutritional recommendations for the prevention and treatment of overweight and obesity in adults (FESNAD-SEEDO consensus document). The role of diet in obesity treatment (III/III). *Nutr Hosp* 2012; **27**: 833-864 [PMID: 23114947 DOI: 10.3305/nh.2012.27.3.5680]
- 24 **Escalante-Pulido M**, Escalante-Herrera A, Milke-Najar ME, Alpizar-Salazar M. Effects of weight loss on insulin secretion and in vivo insulin sensitivity in obese diabetic and non-diabetic subjects. *Diabetes Nutr Metab* 2003; **16**: 277-283 [PMID: 15000438]
  - 25 **Pi-Sunyer X**, Blackburn G, Brancati FL, Bray GA, Bright R, Clark JM, Curtis JM, Espeland MA, Foreyt JP, Graves K, Haffner SM, Harrison B, Hill JO, Horton ES, Jakicic J, Jeffery RW, Johnson KC, Kahn S, Kelley DE, Kitabchi AE, Knowler WC, Lewis CE, Maschak-Carey BJ, Montgomery B, Nathan DM, Patricio J, Peters A, Redmon JB, Reeves RS, Ryan DH, Safford M, Van Dorsten B, Wadden TA, Wagenknecht L, Wesche-Thobaben J, Wing RR, Yanovski SZ. Reduction in weight and cardiovascular disease risk factors in individuals with type 2 diabetes: one-year results of the look AHEAD trial. *Diabetes Care* 2007; **30**: 1374-1383 [PMID: 17363746 DOI: 10.2337/dc07-0048]
  - 26 **Wing RR**, Bolin P, Brancati FL, Bray GA, Clark JM, Coday M, Crow RS, Curtis JM, Egan CM, Espeland MA, Evans M, Foreyt JP, Ghazarian S, Gregg EW, Harrison B, Hazuda HP, Hill JO, Horton ES, Hubbard VS, Jakicic JM, Jeffery RW, Johnson KC, Kahn SE, Kitabchi AE, Knowler WC, Lewis CE, Maschak-Carey BJ, Montez MG, Murillo A, Nathan DM, Patricio J, Peters A, Pi-Sunyer X, Pownall H, Reboussin D, Regensteiner JG, Rickman AD, Ryan DH, Safford M, Wadden TA, Wagenknecht LE, West DS, Williamson DF, Yanovski SZ. Cardiovascular effects of intensive lifestyle intervention in type 2 diabetes. *N Engl J Med* 2013; **369**: 145-154 [PMID: 23796131 DOI: 10.1056/NEJMoa1212914]
  - 27 **Wheeler ML**, Dunbar SA, Jaacks LM, Karmally W, Mayer-Davis EJ, Wylie-Rosett J, Yancy WS. Macronutrients, food groups, and eating patterns in the management of diabetes: a systematic review of the literature, 2010. *Diabetes Care* 2012; **35**: 434-445 [PMID: 22275443 DOI: 10.2337/dc11-2216]
  - 28 **Evert AB**, Boucher JL, Cypress M, Dunbar SA, Franz MJ, Mayer-Davis EJ, Neumiller JJ, Nwankwo R, Verdi CL, Urbanski P, Yancy WS. Nutrition therapy recommendations for the management of adults with diabetes. *Diabetes Care* 2013; **36**: 3821-3842 [PMID: 24107659 DOI: 10.2337/dc13-2042]
  - 29 **Thomas D**, Elliott EJ. Low glycaemic index, or low glycaemic load, diets for diabetes mellitus. *Cochrane Database Syst Rev* 2009; **(1)**: CD006296 [PMID: 19160276 DOI: 10.1002/14651858.CD006296.pub2]
  - 30 **Franz MJ**. Diabetes mellitus nutrition therapy: beyond the glycemic index. *Arch Intern Med* 2012; **172**: 1660-1661 [PMID: 23090061 DOI: 10.1001/2013.jamainternmed.871]
  - 31 **Burger KN**, Beulens JW, van der Schouw YT, Sluijs I, Spijkerman AM, Sluik D, Boeing H, Kaaks R, Teucher B, Dethlefsen C, Overvad K, Tjønneland A, Kyrø C, Barricarte A, Bendinelli B, Krogh V, Tumino R, Sacerdote C, Mattiello A, Nilsson PM, Orho-Melander M, Rolandsson O, Huerta JM, Crowe F, Allen N, Nöthlings U. Dietary fiber, carbohydrate quality and quantity, and mortality risk of individuals with diabetes mellitus. *PLoS One* 2012; **7**: e43127 [PMID: 22927948 DOI: 10.1371/journal.pone.0043127]
  - 32 **Post RE**, Mainous AG, King DE, Simpson KN. Dietary fiber for the treatment of type 2 diabetes mellitus: a meta-analysis. *J Am Board Fam Med* 2012; **25**: 16-23 [PMID: 22218620 DOI: 10.3122/jabfm.2012.01.110148]
  - 33 **Sanz Paris A**, Boj Carceller D, Melchor Lacleta I, Alberio Gamboa R. Sugar and diabetes: international recommendations. *Nutr Hosp* 2013; **28** Suppl 4: 72-80 [PMID: 23834095 DOI: 10.3305/nh.2013.28.sup4.6799]
  - 34 **Stanhope KL**, Schwarz JM, Keim NL, Griffen SC, Bremer AA, Graham JL, Hatcher B, Cox CL, Dyachenko A, Zhang W, McGahan JP, Seibert A, Krauss RM, Chiu S, Schaefer EJ, Ai M, Otokoza S, Nakajima K, Nakano T, Beysen C, Hellerstein MK, Berghlund L, Havel PJ. Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. *J Clin Invest* 2009; **119**: 1322-1334 [PMID: 19381015 DOI: 10.1172/JCI37385]
  - 35 **Serra-Majem L**, Riobó Serván P, Belmonte Cortés S, Anadón Navarro A, Aranceta Bartrina J, Franco Vargas E, García-Closas R, Gómez-Candela C, Herrero Sancho E, La Vecchia C, López Díaz-Ufano ML, Varela-Moreiras G, Vázquez Castro J, Ribas-Barba L, Alcaraz-Cebrián F, García-Luna PP, González-Gomis M, González-Gross M, Granado de la Orden S, López-Sobaler AM, Moreno Villares JM, Ortega Anta RM, Pérez-Rodrigo C, Polanco Allué I, Urrialde de Andrés R. Chinchón declaration; decalogue on low- and no-calorie sweeteners (LNCS). *Nutr Hosp* 2014; **29**: 719-734 [PMID: 24679013 DOI: 10.3305/nh.2014.29.4.7393]
  - 36 **Gannon MC**, Nuttall FQ, Saeed A, Jordan K, Hoover H. An increase in dietary protein improves the blood glucose response in persons with type 2 diabetes. *Am J Clin Nutr* 2003; **78**: 734-741 [PMID: 14522731]
  - 37 **Wycherley TP**, Noakes M, Clifton PM, Cleanthous X, Keogh JB, Brinkworth GD. A high-protein diet with resistance exercise training improves weight loss and body composition in overweight and obese patients with type 2 diabetes. *Diabetes Care* 2010; **33**: 969-976 [PMID: 20150293 DOI: 10.2337/dc09-1974]
  - 38 **Melmed S**, Polonsky KS, Larsen PR, Kronenberg HM. Disorders of Carbohydrate and Metabolism. Williams Textbook of Endocrinology 12th edition. New York: USA Press, 2011: 1413-1414
  - 39 **Vitolins MZ**, Anderson AM, Delahanty L, Raynor H, Miller GD, Mobley C, Reeves R, Yamamoto M, Champagne C, Wing RR, Mayer-Davis E. Action for Health in Diabetes (Look AHEAD) trial: baseline evaluation of selected nutrients and food group intake. *J Am Diet Assoc* 2009; **109**: 1367-1375 [PMID: 19631042 DOI: 10.1016/j.jada.2009.05.016]
  - 40 **Estruch R**, Ros E, Salas-Salvadó J, Covas MI, Corella D, Arós F, Gómez-Gracia E, Ruiz-Gutiérrez V, Fiol M, Lapetra J, Lamuela-Raventós RM, Serra-Majem L, Pintó X, Basora J, Muñoz MA, Sorlí JV, Martínez JA, Martínez-González MA. Primary prevention of cardiovascular disease with a Mediterranean diet. *N Engl J Med* 2013; **368**: 1279-1290 [PMID: 23432189 DOI: 10.1056/NEJMoa1200303]
  - 41 **Bosch J**, Gerstein HC, Dagenais GR, Díaz R, Dyal L, Jung H, Maggiono AP, Probstfield J, Ramachandran A, Riddle MC, Rydén LE, Yusuf S. n-3 fatty acids and cardiovascular outcomes in patients with dysglycemia. *N Engl J Med* 2012; **367**: 309-318 [PMID: 22686415 DOI: 10.1056/NEJMoa1203859]
  - 42 **Karlström BE**, Järvi AE, Byberg L, Berglund LG, Vessby BO. Fatty fish in the diet of patients with type 2 diabetes: comparison of the metabolic effects of foods rich in n-3 and n-6 fatty acids. *Am J Clin Nutr* 2011; **94**: 26-33 [PMID: 21613555 DOI: 10.3945/ajcn.110.006221]
  - 43 Standards of Medical Care in Diabetes-2016: Summary of Revisions. *Diabetes Care* 2016; **39** Suppl 1: S4-S5 [PMID: 26696680 DOI: 10.2337/dc16-S013]
  - 44 **Phielix E**, Meex R, Moonen-Kornips E, Hesselink MK, Schrauwen P. Exercise training increases mitochondrial content and ex vivo mitochondrial function similarly in patients with type 2 diabetes and in control individuals. *Diabetologia* 2010; **53**: 1714-1721 [PMID: 20422397 DOI: 10.1007/s00125-010-1764-2]
  - 45 **Chudyk A**, Petrella RJ. Effects of exercise on cardiovascular risk factors in type 2 diabetes: a meta-analysis. *Diabetes Care* 2011; **34**: 1228-1237 [PMID: 21525503 DOI: 10.2337/dc10-1881]
  - 46 **Sigal RJ**, Kenny GP, Boulé NG, Wells GA, Prud'homme D, Fortier M, Reid RD, Tulloch H, Coyle D, Phillips P, Jennings A, Jaffey J. Effects of aerobic training, resistance training, or both on glycemic control in type 2 diabetes: a randomized trial. *Ann Intern Med* 2007; **147**: 357-369 [PMID: 17876019 DOI: 10.7326/0003-4819-147-6-200709180-00005]
  - 47 **Colberg SR**, Sigal RJ, Fernhall B, Regensteiner JG, Blissmer BJ, Rubin RR, Chasan-Taber L, Albright AL, Braun B. Exercise and type 2 diabetes: the American College of Sports Medicine and the American Diabetes Association: joint position statement. *Diabetes Care* 2010; **33**: e147-e167 [PMID: 21115758 DOI: 10.2337/

- dc10-9990]
- 48 **Armstrong MJ**, Sigal RJ. Exercise as Medicine: Key Concepts in Discussing Physical Activity with Patients who have Type 2 Diabetes. *Can J Diabetes* 2015; **39** Suppl 5: S129-S133 [PMID: 26653253 DOI: 10.1016/j.cjcd.2015.09.081]
  - 49 Exercise prescription for patients with type 2 diabetes-a synthesis of international recommendations: narrative review. *Br J Sports Med* 2015; pii: bjsports-2015-094895 [PMID: 26719499 DOI: 10.1136/bjsports-2015-094895]
  - 50 **Duclos M**, Oppert JM, Verges B, Coliche V, Gautier JF, Guezennec Y, Reach G, Strauch G. Physical activity and type 2 diabetes. Recommendations of the SFD (Francophone Diabetes Society) diabetes and physical activity working group. *Diabetes Metab* 2013; **39**: 205-216 [PMID: 23643351 DOI: 10.1016/j.diabet.2013.03.005]
  - 51 **Balducci S**, Zanuso S, Nicolucci A, De Feo P, Cavallo S, Cardelli P, Fallucca S, Alessi E, Fallucca F, Pugliese G. Effect of an intensive exercise intervention strategy on modifiable cardiovascular risk factors in subjects with type 2 diabetes mellitus: a randomized controlled trial: the Italian Diabetes and Exercise Study (IDES). *Arch Intern Med* 2010; **170**: 1794-1803 [PMID: 21059972 DOI: 10.1001/archinternmed.2010.380]
  - 52 **Balducci S**, Iacobellis G, Parisi L, Di Biase N, Calandriello E, Leonetti F, Fallucca F. Exercise training can modify the natural history of diabetic peripheral neuropathy. *J Diabetes Complications* 2006; **20**: 216-223 [PMID: 16798472 DOI: 10.1016/j.jdiacomp.2005.07.005]
  - 53 **Sigal RJ**, Kenny GP, Wasserman DH, Castaneda-Sceppa C, White RD. Physical activity/exercise and type 2 diabetes: a consensus statement from the American Diabetes Association. *Diabetes Care* 2006; **29**: 1433-1438 [PMID: 16732040 DOI: 10.2337/dc06-9910]
  - 54 **Pagkalos M**, Koutlianos N, Kouidi E, Pagkalos E, Mandroukas K, Deligiannis A. Heart rate variability modifications following exercise training in type 2 diabetic patients with definite cardiac autonomic neuropathy. *Br J Sports Med* 2008; **42**: 47-54 [PMID: 17526623]
  - 55 **Vinik AI**, Ziegler D. Diabetic cardiovascular autonomic neuropathy. *Circulation* 2007; **115**: 387-397 [PMID: 17242296 DOI: 10.1161/CIRCULATIONAHA.106.634949]
  - 56 **Sigal RJ**, Armstrong MJ, Colby P, Kenny GP, Plotnikoff RC, Reichert SM, Riddell MC. Physical activity and diabetes. *Can J Diabetes* 2013; **37** Suppl 1: S40-S44 [PMID: 24070962 DOI: 10.1016/j.cjcd.2013.01.018]
  - 57 **Inzucchi SE**, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, Peters AL, Tsapas A, Wender R, Matthews DR. Management of hyperglycemia in type 2 diabetes: a patient-centered approach: position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care* 2012; **35**: 1364-1379 [PMID: 22517736 DOI: 10.2337/dc12-0413]
  - 58 **An H**, He L. Current understanding of metformin effect on the control of hyperglycemia in diabetes. *J Endocrinol* 2016; **228**: R97-106 [PMID: 26743209 DOI: 10.1530/JOE-15-0447]
  - 59 **Shin NR**, Lee JC, Lee HY, Kim MS, Whon TW, Lee MS, Bae JW. An increase in the *Akkermansia* spp. population induced by metformin treatment improves glucose homeostasis in diet-induced obese mice. *Gut* 2014; **63**: 727-735 [PMID: 23804561 DOI: 10.1136/gutjnl-2012-303839]
  - 60 **Zhou G**, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, Wu M, Ventre J, Doebber T, Fujii N, Musi N, Hirshman MF, Goodyear LJ, Moller DE. Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest* 2001; **108**: 1167-1174 [PMID: 11602624 DOI: 10.1172/JCI13505]
  - 61 **Song R**. Mechanism of Metformin: A Tale of Two Sites. *Diabetes Care* 2016; **39**: 187-189 [PMID: 26798149 DOI: 10.2337/dci15-0013]
  - 62 **Alessi DR**, Sakamoto K, Bayascas JR. LKB1-dependent signaling pathways. *Annu Rev Biochem* 2006; **75**: 137-163 [PMID: 16756488 DOI: 10.1146/annurev.biochem.75.103004.142702]
  - 63 **Lalau JD**, Arnouts P, Sharif A, De Broe ME. Metformin and other antidiabetic agents in renal failure patients. *Kidney Int* 2015; **87**: 308-322 [PMID: 24599253 DOI: 10.1038/ki.2014.19]
  - 64 **Lu WR**, Defilippi J, Braun A. Unleash metformin: reconsideration of the contraindication in patients with renal impairment. *Ann Pharmacother* 2013; **47**: 1488-1497 [PMID: 24259604 DOI: 10.1177/1060028013505428]
  - 65 **Inzucchi SE**, Lipska KJ, Mayo H, Bailey CJ, McGuire DK. Metformin in patients with type 2 diabetes and kidney disease: a systematic review. *JAMA* 2014; **312**: 2668-2675 [PMID: 25536258 DOI: 10.1001/jama.2014.15298]
  - 66 **Salpeter SR**, Greyber E, Pasternak GA, Salpeter EE. Risk of fatal and nonfatal lactic acidosis with metformin use in type 2 diabetes mellitus. *Cochrane Database Syst Rev* 2010; **(4)**: CD002967 [PMID: 20393934 DOI: 10.1002/14651858.CD002967]
  - 67 **Libby G**, Donnelly LA, Donnan PT, Alessi DR, Morris AD, Evans JM. New users of metformin are at low risk of incident cancer: a cohort study among people with type 2 diabetes. *Diabetes Care* 2009; **32**: 1620-1625 [PMID: 19564453 DOI: 10.2337/dc08-2175]
  - 68 **Landman GW**, Kleefstra N, van Hateren KJ, Groenier KH, Gans RO, Bilo HJ. Metformin associated with lower cancer mortality in type 2 diabetes: ZODIAC-16. *Diabetes Care* 2010; **33**: 322-326 [PMID: 19918015 DOI: 10.2337/dc09-1380]
  - 69 **Noto H**, Goto A, Tsujimoto T, Noda M. Cancer risk in diabetic patients treated with metformin: a systematic review and meta-analysis. *PLoS One* 2012; **7**: e33411 [PMID: 22448244 DOI: 10.1371/journal.pone.0033411]
  - 70 **Yin M**, Zhou J, Gorak EJ, Quddus F. Metformin is associated with survival benefit in cancer patients with concurrent type 2 diabetes: a systematic review and meta-analysis. *Oncologist* 2013; **18**: 1248-1255 [PMID: 24258613 DOI: 10.1634/theoncologist.2013-0111]
  - 71 **Eldor R**, Raz I. Diabetes therapy--focus on Asia: second-line therapy debate: insulin/secretagogues. *Diabetes Metab Res Rev* 2012; **28** Suppl 2: 85-89 [PMID: 23280872]
  - 72 **Genuth S**. Should sulfonylureas remain an acceptable first-line add-on to metformin therapy in patients with type 2 diabetes? No, it's time to move on! *Diabetes Care* 2015; **38**: 170-175 [PMID: 25538314]
  - 73 **Gerich J**, Raskin P, Jean-Louis L, Purkayastha D, Baron MA. PRESERVE-beta: two-year efficacy and safety of initial combination therapy with nateglinide or glyburide plus metformin. *Diabetes Care* 2005; **28**: 2093-2099 [PMID: 16123472 DOI: 10.2337/diacare.28.9.2093]
  - 74 **Bryan J**, Crane A, Vila-Carriels WH, Babenko AP, Aguilar-Bryan L. Insulin secretagogues, sulfonylurea receptors and K(ATP) channels. *Curr Pharm Des* 2005; **11**: 2699-2716 [PMID: 16101450 DOI: 10.2174/1381612054546879]
  - 75 **Ferrannini E**, DeFronzo RA. Impact of glucose-lowering drugs on cardiovascular disease in type 2 diabetes. *Eur Heart J* 2015; **36**: 2288-2296 [PMID: 26063450 DOI: 10.1093/eurheartj/ehv239]
  - 76 **Lau DC**, Teoh H. Impact of Current and Emerging Glucose-Lowering Drugs on Body Weight in Type 2 Diabetes. *Can J Diabetes* 2015; **39** Suppl 5: S148-S154 [PMID: 26654858 DOI: 10.1016/j.cjcd.2015.09.090]
  - 77 **Holman RR**, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-year follow-up of intensive glucose control in type 2 diabetes. *N Engl J Med* 2008; **359**: 1577-1589 [PMID: 18784090 DOI: 10.1056/NEJMoa0806470]
  - 78 **Takahashi A**, Nagashima K, Hamasaki A, Kuwamura N, Kawasaki Y, Ikeda H, Yamada Y, Inagaki N, Seino Y. Sulfonylurea and glinide reduce insulin content, functional expression of K(ATP) channels, and accelerate apoptotic beta-cell death in the chronic phase. *Diabetes Res Clin Pract* 2007; **77**: 343-350 [PMID: 17316868 DOI: 10.1016/j.diabres.2006.12.021]
  - 79 **Maedler K**, Carr RD, Bosco D, Zuellig RA, Berney T, Donath MY. Sulfonylurea induced beta-cell apoptosis in cultured human islets. *J Clin Endocrinol Metab* 2005; **90**: 501-506 [PMID: 15483097 DOI: 10.1210/jc.2004-0699]
  - 80 **Kahn SE**, Haffner SM, Heise MA, Herman WH, Holman RR, Jones NP, Kravitz BG, Lachin JM, O'Neill MC, Zinman B, Viberti G. Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. *N Engl J Med* 2006; **355**: 2427-2443 [PMID: 17145742 DOI: 10.1056/NEJMoa066224]
  - 81 Intensive blood-glucose control with sulphonylureas or insulin



- compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. *Lancet* 1998; **352**: 837-853 [PMID: 9742976 DOI: 10.1016/S0140-6736(98)07019-6]
- 82 **McIntosh B**, Cameron C, Singh SR, Yu C, Ahuja T, Welton NJ, Dahl M. Second-line therapy in patients with type 2 diabetes inadequately controlled with metformin monotherapy: a systematic review and mixed-treatment comparison meta-analysis. *Open Med* 2011; **5**: e35-e48 [PMID: 22046219]
  - 83 **Lim PC**, Chong CP. What's next after metformin? focus on sulphonylurea: add-on or combination therapy. *Pharm Pract (Granada)* 2015; **13**: 606 [PMID: 26445623 DOI: 10.18549/PharmPract.2015.03.606]
  - 84 **Scott LJ**. Repaglinide: a review of its use in type 2 diabetes mellitus. *Drugs* 2012; **72**: 249-272 [PMID: 22268393 DOI: 10.2165/11207600-000000000-00000]
  - 85 **Gangji AS**, Cukierman T, Gerstein HC, Goldsmith CH, Clase CM. A systematic review and meta-analysis of hypoglycemia and cardiovascular events: a comparison of glyburide with other secretagogues and with insulin. *Diabetes Care* 2007; **30**: 389-394 [PMID: 17259518 DOI: 10.2337/dc06-1789]
  - 86 **Cryer PE**, Davis SN, Shamon H. Hypoglycemia in diabetes. *Diabetes Care* 2003; **26**: 1902-1912 [PMID: 12766131 DOI: 10.2337/diacare.26.6.1902]
  - 87 **International Hypoglycaemia Study Group**. Minimizing Hypoglycemia in Diabetes. *Diabetes Care* 2015; **38**: 1583-1591 [PMID: 26207052 DOI: 10.2337/dc15-0279]
  - 88 **Morgan CL**, Mukherjee J, Jenkins-Jones S, Holden SE, Currie CJ. Association between first-line monotherapy with sulphonylurea versus metformin and risk of all-cause mortality and cardiovascular events: a retrospective, observational study. *Diabetes Obes Metab* 2014; **16**: 957-962 [PMID: 24720708 DOI: 10.1111/dom.12302]
  - 89 **Jørgensen CH**, Gislason GH, Andersson C, Ahlehoff O, Charlott M, Schramm TK, Vaag A, Abildstrøm SZ, Torp-Pedersen C, Hansen PR. Effects of oral glucose-lowering drugs on long term outcomes in patients with diabetes mellitus following myocardial infarction not treated with emergent percutaneous coronary intervention--a retrospective nationwide cohort study. *Cardiovasc Diabetol* 2010; **9**: 54 [PMID: 20843380 DOI: 10.1186/1475-2840-9-54]
  - 90 **Kalra S**, Gupta Y. Sulfonylureas. *J Pak Med Assoc* 2015; **65**: 101-104 [PMID: 25831689]
  - 91 **Rosak C**, Mertes G. Critical evaluation of the role of acarbose in the treatment of diabetes: patient considerations. *Diabetes Metab Syndr Obes* 2012; **5**: 357-367 [PMID: 23093911 DOI: 10.2147/DMSO.S28340]
  - 92 **van de Laar FA**, Lucassen PL, Akkermans RP, van de Lisdonk EH, Rutten GE, van Weel C. Alpha-glucosidase inhibitors for patients with type 2 diabetes: results from a Cochrane systematic review and meta-analysis. *Diabetes Care* 2005; **28**: 154-163 [PMID: 15616251 DOI: 10.2337/diacare.28.1.154]
  - 93 **Chiasson JL**, Gomis R, Hanefeld M, Josse RG, Karasik A, Laakso M. The STOP-NIDDM Trial: an international study on the efficacy of an alpha-glucosidase inhibitor to prevent type 2 diabetes in a population with impaired glucose tolerance: rationale, design, and preliminary screening data. Study to Prevent Non-Insulin-Dependent Diabetes Mellitus. *Diabetes Care* 1998; **21**: 1720-1725 [PMID: 9773737 DOI: 10.2337/diacare.21.10.1720]
  - 94 **Chiasson JL**, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M. Acarbose treatment and the risk of cardiovascular disease and hypertension in patients with impaired glucose tolerance: the STOP-NIDDM trial. *JAMA* 2003; **290**: 486-494 [PMID: 12876091 DOI: 10.1001/jama.290.4.486]
  - 95 **Abe M**, Okada K, Soma M. Antidiabetic agents in patients with chronic kidney disease and end-stage renal disease on dialysis: metabolism and clinical practice. *Curr Drug Metab* 2011; **12**: 57-69 [PMID: 21303332 DOI: 10.2174/138920011794520053]
  - 96 **Kao CC**, Wu PC, Wu CH, Chen LK, Chen HH, Wu MS, Wu VC. Risk of liver injury after  $\alpha$ -glucosidase inhibitor therapy in advanced chronic kidney disease patients. *Sci Rep* 2016; **6**: 18996 [PMID: 26751038]
  - 97 **Park KS**, Ciaraldi TP, Abrams-Carter L, Mudaliar S, Nikoulina SE, Henry RR. PPAR- $\gamma$  gene expression is elevated in skeletal muscle of obese and type II diabetic subjects. *Diabetes* 1997; **46**: 1230-1234 [PMID: 9200661 DOI: 10.2337/diab.46.7.1230]
  - 98 **Ryan KK**, Li B, Grayson BE, Matter EK, Woods SC, Seeley RJ. A role for central nervous system PPAR- $\gamma$  in the regulation of energy balance. *Nat Med* 2011; **17**: 623-626 [PMID: 21532595 DOI: 10.1038/nm.2349]
  - 99 **Bogacka I**, Xie H, Bray GA, Smith SR. The effect of pioglitazone on peroxisome proliferator-activated receptor- $\gamma$  target genes related to lipid storage in vivo. *Diabetes Care* 2004; **27**: 1660-1667 [PMID: 15220243 DOI: 10.2337/diacare.27.7.1660]
  - 100 **Guan Y**, Hao C, Cha DR, Rao R, Lu W, Kohan DE, Magnuson MA, Redha R, Zhang Y, Breyer MD. Thiazolidinediones expand body fluid volume through PPAR $\gamma$  stimulation of ENaC-mediated renal salt absorption. *Nat Med* 2005; **11**: 861-866 [PMID: 16007095 DOI: 10.1038/nm1278]
  - 101 **Nesto RW**, Bell D, Bonow RO, Fonseca V, Grundy SM, Horton ES, Le Winter M, Porte D, Semenkovich CF, Smith S, Young LH, Kahn R. Thiazolidinedione use, fluid retention, and congestive heart failure: a consensus statement from the American Heart Association and American Diabetes Association. October 7, 2003. *Circulation* 2003; **108**: 2941-2948 [PMID: 14662691 DOI: 10.1161/01.CIR.0000103683.99399.7E]
  - 102 **Dormandy J**, Bhattacharya M, van Troostenburg de Bruyn AR. Safety and tolerability of pioglitazone in high-risk patients with type 2 diabetes: an overview of data from PROactive. *Drug Saf* 2009; **32**: 187-202 [PMID: 19338377 DOI: 10.2165/00002018-20093203-0-00002]
  - 103 **Lewis JD**, Ferrara A, Peng T, Hedderston M, Bilker WB, Quesenberry CP, Vaughn DJ, Nessel L, Selby J, Strom BL. Risk of bladder cancer among diabetic patients treated with pioglitazone: interim report of a longitudinal cohort study. *Diabetes Care* 2011; **34**: 916-922 [PMID: 21447663 DOI: 10.2337/dc10-1068]
  - 104 **Piccinni C**, Motola D, Marchesini G, Poluzzi E. Assessing the association of pioglitazone use and bladder cancer through drug adverse event reporting. *Diabetes Care* 2011; **34**: 1369-1371 [PMID: 21515844 DOI: 10.2337/dc10-2412]
  - 105 **Lecka-Czernik B**, Ackert-Bicknell C, Adamo ML, Marmolejos V, Churchill GA, Shockley KR, Reid IR, Grey A, Rosen CJ. Activation of peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) by rosiglitazone suppresses components of the insulin-like growth factor regulatory system in vitro and in vivo. *Endocrinology* 2007; **148**: 903-911 [PMID: 17122083 DOI: 10.1210/en.2006-1121]
  - 106 **Watkins PB**, Whitcomb RW. Hepatic dysfunction associated with troglitazone. *N Engl J Med* 1998; **338**: 916-917 [PMID: 9518284 DOI: 10.1056/NEJM199803263381314]
  - 107 **Bergman AJ**, Cote J, Yi B, Marbury T, Swan SK, Smith W, Gottesdiener K, Wagner J, Herman GA. Effect of renal insufficiency on the pharmacokinetics of sitagliptin, a dipeptidyl peptidase-4 inhibitor. *Diabetes Care* 2007; **30**: 1862-1864 [PMID: 17468348 DOI: 10.2337/dc06-2545]
  - 108 **Raz I**, Hanefeld M, Xu L, Caria C, Williams-Herman D, Khatami H. Efficacy and safety of the dipeptidyl peptidase-4 inhibitor sitagliptin as monotherapy in patients with type 2 diabetes mellitus. *Diabetologia* 2006; **49**: 2564-2571 [PMID: 17001471 DOI: 10.1007/s00125-006-0416-z]
  - 109 **Nauck MA**, Meininger G, Sheng D, Terranella L, Stein PP. Efficacy and safety of the dipeptidyl peptidase-4 inhibitor, sitagliptin, compared with the sulfonylurea, glipizide, in patients with type 2 diabetes inadequately controlled on metformin alone: a randomized, double-blind, non-inferiority trial. *Diabetes Obes Metab* 2007; **9**: 194-205 [PMID: 17300595 DOI: 10.1111/j.1463-1326.2006.00704.x]
  - 110 **Bosi E**, Camisasca RP, Collober C, Rochotte E, Garber AJ. Effects of vildagliptin on glucose control over 24 weeks in patients with type 2 diabetes inadequately controlled with metformin. *Diabetes Care* 2007; **30**: 890-895 [PMID: 17277036 DOI: 10.2337/dc06-1732]
  - 111 **Rosenstock J**, Sankoh S, List JF. Glucose-lowering activity of the



- dipeptidyl peptidase-4 inhibitor saxagliptin in drug-naïve patients with type 2 diabetes. *Diabetes Obes Metab* 2008; **10**: 376-386 [PMID: 18355324 DOI: 10.1111/j.1463-1326.2008.00876.x]
- 112 **Rosenstock J**, Aguilar-Salinas C, Klein E, Nepal S, List J, Chen R. Effect of saxagliptin monotherapy in treatment-naïve patients with type 2 diabetes. *Curr Med Res Opin* 2009; **25**: 2401-2411 [PMID: 19650754 DOI: 10.1185/03007990903178735]
  - 113 **DeFronzo RA**, Hissa MN, Garber AJ, Luiz Gross J, Yuyan Duan R, Ravichandran S, Chen RS. The efficacy and safety of saxagliptin when added to metformin therapy in patients with inadequately controlled type 2 diabetes with metformin alone. *Diabetes Care* 2009; **32**: 1649-1655 [PMID: 19478198 DOI: 10.2337/dc08-1984]
  - 114 **Del Prato S**, Barnett AH, Huisman H, Neubacher D, Woerle HJ, Dugi KA. Effect of linagliptin monotherapy on glycaemic control and markers of  $\beta$ -cell function in patients with inadequately controlled type 2 diabetes: a randomized controlled trial. *Diabetes Obes Metab* 2011; **13**: 258-267 [PMID: 21205122 DOI: 10.1111/j.1463-1326.2010.01350.x]
  - 115 **Owens DR**, Swallow R, Dugi KA, Woerle HJ. Efficacy and safety of linagliptin in persons with type 2 diabetes inadequately controlled by a combination of metformin and sulphonylurea: a 24-week randomized study. *Diabet Med* 2011; **28**: 1352-1361 [PMID: 21781152 DOI: 10.1111/j.1464-5491.2011.03387.x]
  - 116 **Fujii Y**, Abe M, Higuchi T, Mizuno M, Suzuki H, Matsumoto S, Ito M, Maruyama N, Okada K, Soma M. The dipeptidyl peptidase-4 inhibitor alogliptin improves glycaemic control in type 2 diabetic patients undergoing hemodialysis. *Expert Opin Pharmacother* 2013; **14**: 259-267 [PMID: 23289982 DOI: 10.1517/14656566.2013.761690]
  - 117 **Seino Y**, Miyata Y, Hiroi S, Hirayama M, Kaku K. Efficacy and safety of alogliptin added to metformin in Japanese patients with type 2 diabetes: a randomized, double-blind, placebo-controlled trial with an open-label, long-term extension study. *Diabetes Obes Metab* 2012; **14**: 927-936 [PMID: 22583697 DOI: 10.1111/j.1463-1326.2012.01620.x]
  - 118 **Pratley RE**, Kipnes MS, Fleck PR, Wilson C, Mekki Q. Efficacy and safety of the dipeptidyl peptidase-4 inhibitor alogliptin in patients with type 2 diabetes inadequately controlled by glyburide monotherapy. *Diabetes Obes Metab* 2009; **11**: 167-176 [PMID: 19125778 DOI: 10.1111/j.1463-1326.2008.01016.x]
  - 119 **Rosenstock J**, Rendell MS, Gross JL, Fleck PR, Wilson CA, Mekki Q. Alogliptin added to insulin therapy in patients with type 2 diabetes reduces HbA(1c) without causing weight gain or increased hypoglycaemia. *Diabetes Obes Metab* 2009; **11**: 1145-1152 [PMID: 19758359 DOI: 10.1111/j.1463-1326.2009.01124.x]
  - 120 **Garg R**, Chen W, Pendergrass M. Acute pancreatitis in type 2 diabetes treated with exenatide or sitagliptin: a retrospective observational pharmacy claims analysis. *Diabetes Care* 2010; **33**: 2349-2354 [PMID: 20682680 DOI: 10.2337/dc10-0482]
  - 121 **Singh S**, Chang HY, Richards TM, Weiner JP, Clark JM, Segal JB. Glucagonlike peptide 1-based therapies and risk of hospitalization for acute pancreatitis in type 2 diabetes mellitus: a population-based matched case-control study. *JAMA Intern Med* 2013; **173**: 534-539 [PMID: 23440284 DOI: 10.1001/jamainternmed.2013.2720]
  - 122 **Monami M**, Dicembrini I, Mannucci E. Dipeptidyl peptidase-4 inhibitors and pancreatitis risk: a meta-analysis of randomized clinical trials. *Diabetes Obes Metab* 2014; **16**: 48-56 [PMID: 23837679 DOI: 10.1111/dom.12176]
  - 123 **Thomsen RW**, Pedersen L, Møller N, Kahlert J, Beck-Nielsen H, Sørensen HT. Incretin-based therapy and risk of acute pancreatitis: a nationwide population-based case-control study. *Diabetes Care* 2015; **38**: 1089-1098 [PMID: 25633664 DOI: 10.2337/dc13-2983]
  - 124 **Amori RE**, Lau J, Pittas AG. Efficacy and safety of incretin therapy in type 2 diabetes: systematic review and meta-analysis. *JAMA* 2007; **298**: 194-206 [PMID: 17622601 DOI: 10.1001/jama.298.2.194]
  - 125 **Goßner K**, Gräber S. Longer term safety of dipeptidyl peptidase-4 inhibitors in patients with type 2 diabetes mellitus: systematic review and meta-analysis. *Diabetes Obes Metab* 2012; **14**: 1061-1072 [PMID: 22519906 DOI: 10.1111/j.1463-1326.2012.01610.x]
  - 126 **Charbonnel B**, Karasik A, Liu J, Wu M, Meininger G. Efficacy and safety of the dipeptidyl peptidase-4 inhibitor sitagliptin added to ongoing metformin therapy in patients with type 2 diabetes inadequately controlled with metformin alone. *Diabetes Care* 2006; **29**: 2638-2643 [PMID: 17130197 DOI: 10.2337/dc06-0706]
  - 127 **Green JB**, Bethel MA, Armstrong PW, Buse JB, Engel SS, Garg J, Josse R, Kaufman KD, Koglin J, Korn S, Lachin JM, McGuire DK, Pencina MJ, Standl E, Stein PP, Suryawanshi S, Van de Werf F, Peterson ED, Holman RR. Effect of Sitagliptin on Cardiovascular Outcomes in Type 2 Diabetes. *N Engl J Med* 2015; **373**: 232-242 [PMID: 26052984 DOI: 10.1056/NEJMoa1501352]
  - 128 **Scirica BM**, Bhatt DL, Braunwald E, Steg PG, Davidson J, Hirshberg B, Ohman P, Frederick R, Wiviott SD, Hoffman EB, Cavender MA, Udell JA, Desai NR, Mosenzon O, McGuire DK, Ray KK, Leiter LA, Raz I. Saxagliptin and cardiovascular outcomes in patients with type 2 diabetes mellitus. *N Engl J Med* 2013; **369**: 1317-1326 [PMID: 23992601 DOI: 10.1056/NEJMoa1307684]
  - 129 **Bhatt DL**, Cavender MA. Do dipeptidyl peptidase-4 inhibitors increase the risk of heart failure? *JACC Heart Fail* 2014; **2**: 583-585 [PMID: 24998081 DOI: 10.1016/j.jchf.2014.05.005]
  - 130 **Udell JA**, Cavender MA, Bhatt DL, Chatterjee S, Farkouh ME, Scirica BM. Glucose-lowering drugs or strategies and cardiovascular outcomes in patients with or at risk for type 2 diabetes: a meta-analysis of randomised controlled trials. *Lancet Diabetes Endocrinol* 2015; **3**: 356-366 [PMID: 25791290 DOI: 10.1016/S2213-8587(15)00044-3]
  - 131 **Clifton P**. Do dipeptidyl peptidase IV (DPP-IV) inhibitors cause heart failure? *Clin Ther* 2014; **36**: 2072-2079 [PMID: 25453730 DOI: 10.1016/j.clinthera.2014.10.009]
  - 132 **White WB**, Cannon CP, Heller SR, Nissen SE, Bergenstal RM, Bakris GL, Perez AT, Fleck PR, Mehta CR, Kupfer S, Wilson C, Cushman WC, Zannad F. Alogliptin after acute coronary syndrome in patients with type 2 diabetes. *N Engl J Med* 2013; **369**: 1327-1335 [PMID: 23992602 DOI: 10.1056/NEJMoa1305889]
  - 133 **Zannad F**, Cannon CP, Cushman WC, Bakris GL, Menon V, Perez AT, Fleck PR, Mehta CR, Kupfer S, Wilson C, Lam H, White WB. Heart failure and mortality outcomes in patients with type 2 diabetes taking alogliptin versus placebo in EXAMINE: a multicentre, randomised, double-blind trial. *Lancet* 2015; **385**: 2067-2076 [PMID: 25765696 DOI: 10.1016/S0140-6736(14)62225-X]
  - 134 **Weir MR**. The kidney and type 2 diabetes mellitus: therapeutic implications of SGLT2 inhibitors. *Postgrad Med* 2016; **128**: 290-298 [PMID: 26821720 DOI: 10.1080/00325481.2016.1147926]
  - 135 **Kalra S**. Sodium Glucose Co-Transporter-2 (SGLT2) Inhibitors: A Review of Their Basic and Clinical Pharmacology. *Diabetes Ther* 2014; **5**: 355-366 [PMID: 25424969 DOI: 10.1007/s133-00-014-0089-4]
  - 136 **Wright EM**. Renal Na(+)-glucose cotransporters. *Am J Physiol Renal Physiol* 2001; **280**: F10-F18 [PMID: 11133510]
  - 137 **Lee YJ**, Lee YJ, Han HJ. Regulatory mechanisms of Na(+)/glucose cotransporters in renal proximal tubule cells. *Kidney Int Suppl* 2007; **(106)**: S27-S35 [PMID: 17653207]
  - 138 **Hummel CS**, Lu C, Loo DD, Hirayama BA, Voss AA, Wright EM. Glucose transport by human renal Na+/D-glucose cotransporters SGLT1 and SGLT2. *Am J Physiol Cell Physiol* 2011; **300**: C14-C21 [PMID: 20980548 DOI: 10.1152/ajpcell.00388.2010]
  - 139 **Nauck MA**. Update on developments with SGLT2 inhibitors in the management of type 2 diabetes. *Drug Des Devel Ther* 2014; **8**: 1335-1380 [PMID: 25246775 DOI: 10.2147/DDDT.S50773]
  - 140 **Marsenic O**. Glucose control by the kidney: an emerging target in diabetes. *Am J Kidney Dis* 2009; **53**: 875-883 [PMID: 19324482 DOI: 10.1053/j.ajkd.2008.12.031]
  - 141 **Bailey CJ**, Gross JL, Pieters A, Bastien A, List JF. Effect of dapagliflozin in patients with type 2 diabetes who have inadequate glycaemic control with metformin: a randomised, double-blind, placebo-controlled trial. *Lancet* 2010; **375**: 2223-2233 [PMID: 20609968 DOI: 10.1016/S0140-6736(10)60407-2]
  - 142 **Rosenstock J**, Vico M, Wei L, Salsali A, List JF. Effects of dapagliflozin, an SGLT2 inhibitor, on HbA(1c), body weight, and hypoglycemia risk in patients with type 2 diabetes inadequately

- controlled on pioglitazone monotherapy. *Diabetes Care* 2012; **35**: 1473-1478 [PMID: 22446170 DOI: 10.2337/dc11-1693]
- 143 **Strojek K**, Yoon KH, Hrubá V, Elze M, Langkilde AM, Parikh S. Effect of dapagliflozin in patients with type 2 diabetes who have inadequate glycaemic control with glimepiride: a randomized, 24-week, double-blind, placebo-controlled trial. *Diabetes Obes Metab* 2011; **13**: 928-938 [PMID: 21672123 DOI: 10.1111/j.1463-1326.2011.01434.x]
  - 144 **Wilding JP**, Woo V, Soler NG, Pahor A, Sugg J, Rohwedder K, Parikh S. Long-term efficacy of dapagliflozin in patients with type 2 diabetes mellitus receiving high doses of insulin: a randomized trial. *Ann Intern Med* 2012; **156**: 405-415 [PMID: 22431673 DOI: 10.7326/0003-4819-156-6-201203200-00003]
  - 145 **Bailey CJ**, Gross JL, Hennicken D, Iqbal N, Mansfield TA, List JF. Dapagliflozin add-on to metformin in type 2 diabetes inadequately controlled with metformin: a randomized, double-blind, placebo-controlled 102-week trial. *BMC Med* 2013; **11**: 43 [PMID: 23425012 DOI: 10.1186/1741-7015-11-43]
  - 146 **Nauck MA**, Del Prato S, Meier JJ, Durán-García S, Rohwedder K, Elze M, Parikh SJ. Dapagliflozin versus glipizide as add-on therapy in patients with type 2 diabetes who have inadequate glycemic control with metformin: a randomized, 52-week, double-blind, active-controlled noninferiority trial. *Diabetes Care* 2011; **34**: 2015-2022 [PMID: 21816980 DOI: 10.2337/dc11-0606]
  - 147 **Stenlöf K**, Cefalu WT, Kim KA, Alba M, Usiskin K, Tong C, Canovatchel W, Meininger G. Efficacy and safety of canagliflozin monotherapy in subjects with type 2 diabetes mellitus inadequately controlled with diet and exercise. *Diabetes Obes Metab* 2013; **15**: 372-382 [PMID: 23279307 DOI: 10.1111/dom.12054]
  - 148 **Lavalle-González FJ**, Januszewicz A, Davidson J, Tong C, Qiu R, Canovatchel W, Meininger G. Efficacy and safety of canagliflozin compared with placebo and sitagliptin in patients with type 2 diabetes on background metformin monotherapy: a randomised trial. *Diabetologia* 2013; **56**: 2582-2592 [PMID: 24026211 DOI: 10.1007/s00125-013-3039-1]
  - 149 **Cefalu WT**, Leiter LA, Yoon KH, Arias P, Niskanen L, Xie J, Balis DA, Canovatchel W, Meininger G. Efficacy and safety of canagliflozin versus glimepiride in patients with type 2 diabetes inadequately controlled with metformin (CANTATA-SU): 52 week results from a randomised, double-blind, phase 3 non-inferiority trial. *Lancet* 2013; **382**: 941-950 [PMID: 23850055 DOI: 10.1185/03007995.2013.850066]
  - 150 **Leiter LA**, Langslet G, Cefalu WT, Yoon KH, Arias P, Xie J, Balis D, Millington D, Vercruyse F, Carnovatchel W, Meininger G. Canagliflozin demonstrates durable glycemic improvements over 104 weeks compared with glimepiride in subjects with type 2 diabetes mellitus on metformin. *Can J Diabetes* 2013; **37**: S27 [DOI: 10.1016/j.jcjd.2013.08.081]
  - 151 **Ferrannini E**, Seman LJ, Seewaldt-Becker E. The potent and highly selective sodium-glucose co-transporter (SGLT-2) inhibitor BI10773 is safe and efficacious as monotherapy in patients with type 2 diabetes mellitus. 46th Ann Mtg of the European Association for the Study of Diabetes (EASD), Stockholm, 20-24 Sep 2010. *Diabetologia* 2010; **53** (Suppl 1): S351
  - 152 **Woerle H**, Ferrannini E, Berk A, Hantel S, Pinnett S, Broedl U. Safety and Efficacy of Empagliflozin as Monotherapy or Add-On to Metformin in a 78-Week Open-Label Extension Study in Patients with Type 2 Diabetes. Presented at 72nd American Diabetes Association Scientific Sessions; Philadelphia, PA, USA, June 8-12, 2012
  - 153 **Ferrannini E**, Berk A, Hantel S, Pinnett S, Hach T, Woerle HJ, Broedl UC. Long-term safety and efficacy of empagliflozin, sitagliptin, and metformin: an active-controlled, parallel-group, randomized, 78-week open-label extension study in patients with type 2 diabetes. *Diabetes Care* 2013; **36**: 4015-4021 [PMID: 24186878 DOI: 10.2337/dc13-0663]
  - 154 **Rosenstock J**, Jelaska A, Wang F, Kim G, Broedl U, Woerle HJ, Bajaj HS. Empagliflozin as add-on to basal insulin for 78 weeks improves glycemic control with weight loss in insulin-treated type 2 diabetes. *Can J Diabetes* 2013; **37**: S32 [DOI: 10.1016/j.jcjd.2013.08.093]
  - 155 **Rosenwasser RF**, Sultan S, Sutton D, Choksi R, Epstein BJ. SGLT-2 inhibitors and their potential in the treatment of diabetes. *Diabetes Metab Syndr Obes* 2013; **6**: 453-467 [PMID: 24348059 DOI: 10.2147/DMSO.S34416]
  - 156 Dapagliflozin [summary of product characteristics] Middlesex United Kingdom: Bristol-Myers Squibb/AstraZeneca, 2013
  - 157 **Lambers Heerspink HJ**, de Zeeuw D, Wie L, Leslie B, List J. Dapagliflozin a glucose-regulating drug with diuretic properties in subjects with type 2 diabetes. *Diabetes Obes Metab* 2013; **15**: 853-862 [PMID: 23668478 DOI: 10.1111/dom.12127]
  - 158 **Foote C**, Perkovic V, Neal B. Effects of SGLT2 inhibitors on cardiovascular outcomes. *Diab Vasc Dis Res* 2012; **9**: 117-123 [PMID: 22381403 DOI: 10.1177/1479164112441190]
  - 159 **List JF**, Woo V, Morales E, Tang W, Fiedorek FT. Sodium-glucose cotransport inhibition with dapagliflozin in type 2 diabetes. *Diabetes Care* 2009; **32**: 650-657 [PMID: 19114612 DOI: 10.2337/dc08-1863]
  - 160 **Sha S**, Devineni D, Ghosh A, Polidori D, Hompesch M, Arnolds S, Morrow L, Spitzer H, Demarest K, Rothenberg P. Pharmacodynamic effects of canagliflozin, a sodium glucose co-transporter 2 inhibitor, from a randomized study in patients with type 2 diabetes. *PLoS One* 2014; **9**: e105638 [PMID: 25166023 DOI: 10.1371/journal.pone.0105638]
  - 161 **Ferrannini E**, Ramos SJ, Salsali A, Tang W, List JF. Dapagliflozin monotherapy in type 2 diabetic patients with inadequate glycemic control by diet and exercise: a randomized, double-blind, placebo-controlled, phase 3 trial. *Diabetes Care* 2010; **33**: 2217-2224 [PMID: 20566676 DOI: 10.2337/dc10-0612]
  - 162 **Bolinder J**, Junggren Ö, Johansson L, Wilding J, Langkilde AM, Sjöström CD, Sugg J, Parikh S. Dapagliflozin maintains glycaemic control while reducing weight and body fat mass over 2 years in patients with type 2 diabetes mellitus inadequately controlled on metformin. *Diabetes Obes Metab* 2014; **16**: 159-169 [PMID: 23906445 DOI: 10.1111/dom.12189]
  - 163 **Ruggenenti P**, Porrini EL, Gaspari F, Motterlini N, Cannata A, Carrara F, Cella C, Ferrari S, Stucchi N, Parvanova A, Iliev I, Dodesini AR, Trevisan R, Bossi A, Zaletel J, Remuzzi G. Glomerular hyperfiltration and renal disease progression in type 2 diabetes. *Diabetes Care* 2012; **35**: 2061-2068 [PMID: 22773704 DOI: 10.2337/dc11-2189]
  - 164 **Sarnoski-Brocovich S**, Hilas O. Canagliflozin (invokana), a novel oral agent for type-2 diabetes. *P T* 2013; **38**: 656-666 [PMID: 24391386]
  - 165 **Johansson KM**, Ptaszynska A, Schmitz B, Sugg J, Parikh SJ, List JF. Vulvovaginitis and balanitis in patients with diabetes treated with dapagliflozin. *J Diabetes Complications* 2013; **27**: 479-484 [PMID: 23806570 DOI: 10.1016/j.jdiacomp.2013.04.012]
  - 166 **Ptaszynska A**, Johansson KM, Parikh SJ, de Bruin TW, Apanovitch AM, List JF. Safety profile of dapagliflozin for type 2 diabetes: pooled analysis of clinical studies for overall safety and rare events. *Drug Saf* 2014; **37**: 815-829 [PMID: 25096959 DOI: 10.1007/s40264-014-0213-4]
  - 167 **Kalra S**, Baruah MP, Sahay R. Medication counselling with sodium glucose transporter 2 inhibitor therapy. *Indian J Endocrinol Metab* 2014; **18**: 597-599 [PMID: 25285273 DOI: 10.4103/2230-8210.139206]
  - 168 **Modi A**, Agrawal A, Morgan F. Euglycemic Diabetic Ketoacidosis. *Curr Diabetes Rev* 2016; Epub ahead of print [PMID: 27097605]
  - 169 **Ogawa W**, Sakaguchi K. Euglycemic diabetic ketoacidosis induced by SGLT2 inhibitors: possible mechanism and contributing factors. *J Diabetes Investig* 2016; **7**: 135-138 [PMID: 27042263 DOI: 10.1111/jdi.12401]
  - 170 **Scheen AJ**. EMPA-REG OUTCOME: Empagliflozin reduces mortality in patients with type 2 diabetes at high cardiovascular risk. *Rev Med Liege* 2015; **70**: 583-589 [PMID: 26738271]
  - 171 **Nauck MA**, Homberger E, Siegel EG, Allen RC, Eaton RP, Ebert R, Creutzfeldt W. Incretin effects of increasing glucose loads in man calculated from venous insulin and C-peptide responses. *J Clin Endocrinol Metab* 1986; **63**: 492-498 [PMID: 3522621 DOI: 10.1210/erem.63.4.492]

- 10.1210/jcem-63-2-492]
- 172 **Willms B**, Werner J, Holst JJ, Orskov C, Creutzfeldt W, Nauck MA. Gastric emptying, glucose responses, and insulin secretion after a liquid test meal: effects of exogenous glucagon-like peptide-1 (GLP-1)-(7-36) amide in type 2 (noninsulin-dependent) diabetic patients. *J Clin Endocrinol Metab* 1996; **81**: 327-332 [PMID: 8550773 DOI: 10.1210/jcem.81.1.8550773]
- 173 **Bose AK**, Mocanu MM, Carr RD, Brand CL, Yellon DM. Glucagon-like peptide 1 can directly protect the heart against ischemia/reperfusion injury. *Diabetes* 2005; **54**: 146-151 [PMID: 15616022 DOI: 10.2337/diabetes.54.1.146]
- 174 **Thrainsdottir I**, Malmberg K, Olsson A, Gutniak M, Rydén L. Initial experience with GLP-1 treatment on metabolic control and myocardial function in patients with type 2 diabetes mellitus and heart failure. *Diab Vasc Dis Res* 2004; **1**: 40-43 [PMID: 16305055 DOI: 10.3132/dvdr.2004.005]
- 175 **Yu M**, Moreno C, Hoagland KM, Dahly A, Ditter K, Mistry M, Roman RJ. Antihypertensive effect of glucagon-like peptide 1 in Dahl salt-sensitive rats. *J Hypertens* 2003; **21**: 1125-1135 [PMID: 12777949]
- 176 **Gutzwiller JP**, Tschopp S, Bock A, Zehnder CE, Huber AR, Kreyenbuehl M, Gutmann H, Drewe J, Henzen C, Goeke B, Beglinger C. Glucagon-like peptide 1 induces natriuresis in healthy subjects and in insulin-resistant obese men. *J Clin Endocrinol Metab* 2004; **89**: 3055-3061 [PMID: 15181098 DOI: 10.1210/jc.2003-031403]
- 177 **Göke R**, Fehmann HC, Linn T, Schmidt H, Krause M, Eng J, Göke B. Exendin-4 is a high potency agonist and truncated exendin-(9-39)-amide an antagonist at the glucagon-like peptide 1-(7-36)-amide receptor of insulin-secreting beta-cells. *J Biol Chem* 1993; **268**: 19650-19655 [PMID: 8396143]
- 178 **DeYoung MB**, MacConell L, Sarin V, Trautmann M, Herbert P. Encapsulation of exenatide in poly-(D,L-lactide-co-glycolide) microspheres produced an investigational long-acting once-weekly formulation for type 2 diabetes. *Diabetes Technol Ther* 2011; **13**: 1145-1154 [PMID: 21751887 DOI: 10.1089/dia.2011.0050]
- 179 **Quianzon CCL**, Shomal ME. Lixisenatide-once-daily glucagon-like peptide-1 receptor agonist in the management of type 2 diabetes. *Eur Endocrinol* 2012; **8**: 12-17 [DOI: 10.17925/EE.2012.08.01.12]
- 180 **Buse JB**, Henry RR, Han J, Kim DD, Fineman MS, Baron AD. Effects of exenatide (exendin-4) on glycemic control over 30 weeks in sulfonylurea-treated patients with type 2 diabetes. *Diabetes Care* 2004; **27**: 2628-2635 [PMID: 15504997 DOI: 10.2337/diacare.27.11.2628]
- 181 **DeFronzo RA**, Ratner RE, Han J, Kim DD, Fineman MS, Baron AD. Effects of exenatide (exendin-4) on glycemic control and weight over 30 weeks in metformin-treated patients with type 2 diabetes. *Diabetes Care* 2005; **28**: 1092-1100 [PMID: 15855572 DOI: 10.2337/diacare.28.5.1092]
- 182 **Kendall DM**, Riddle MC, Rosenstock J, Zhuang D, Kim DD, Fineman MS, Baron AD. Effects of exenatide (exendin-4) on glycemic control over 30 weeks in patients with type 2 diabetes treated with metformin and a sulfonylurea. *Diabetes Care* 2005; **28**: 1083-1091 [PMID: 15855571 DOI: 10.2337/diacare.28.5.1083]
- 183 **Zinman B**, Hoogwerf BJ, Durán García S, Milton DR, Giaconia JM, Kim DD, Trautmann ME, Brodows RG. The effect of adding exenatide to a thiazolidinedione in suboptimally controlled type 2 diabetes: a randomized trial. *Ann Intern Med* 2007; **146**: 477-485 [PMID: 17404349 DOI: 10.7326/0003-4819-146-7-200704030-00003]
- 184 **Heine RJ**, Van Gaal LF, Johns D, Mihm MJ, Widel MH, Brodows RG. Exenatide versus insulin glargine in patients with suboptimally controlled type 2 diabetes: a randomized trial. *Ann Intern Med* 2005; **143**: 559-569 [PMID: 16230722 DOI: 10.7326/0003-4819-143-8-200501180-00006]
- 185 **Nauck MA**, Duran S, Kim D, Johns D, Northrup J, Festa A, Brodows R, Trautmann M. A comparison of twice-daily exenatide and biphasic insulin aspart in patients with type 2 diabetes who were suboptimally controlled with sulfonylurea and metformin: a non-inferiority study. *Diabetologia* 2007; **50**: 259-267 [PMID: 17160407 DOI: 10.1007/s00125-006-0510-2]
- 186 **Ahrén B**, Leguizamo Dimas A, Miossec P, Saubadu S, Aronson R. Efficacy and safety of lixisenatide once-daily morning or evening injections in type 2 diabetes inadequately controlled on metformin (GetGoal-M). *Diabetes Care* 2013; **36**: 2543-2550 [PMID: 23536584 DOI: 10.2337/dc12-2006]
- 187 **Rosenstock J**, Raccach D, Korányi L, Maffei L, Boka G, Miossec P, Gerich JE. Efficacy and safety of lixisenatide once daily versus exenatide twice daily in type 2 diabetes inadequately controlled on metformin: a 24-week, randomized, open-label, active-controlled study (GetGoal-X). *Diabetes Care* 2013; **36**: 2945-2951 [PMID: 23698396 DOI: 10.2337/dc12-2709]
- 188 **Riddle MC**, Forst T, Aronson R, Sauque-Reyna L, Souhami E, Silvestre L, Ping L, Rosenstock J. Adding once-daily lixisenatide for type 2 diabetes inadequately controlled with newly initiated and continuously titrated basal insulin glargine: a 24-week, randomized, placebo-controlled study (GetGoal-Duo 1). *Diabetes Care* 2013; **36**: 2497-2503 [PMID: 23564915 DOI: 10.2337/dc12-2462]
- 189 **Riddle MC**, Aronson R, Home P, Marre M, Niemoeller E, Miossec P, Ping L, Ye J, Rosenstock J. Adding once-daily lixisenatide for type 2 diabetes inadequately controlled by established basal insulin: a 24-week, randomized, placebo-controlled comparison (GetGoal-L). *Diabetes Care* 2013; **36**: 2489-2496 [PMID: 23628617 DOI: 10.2337/dc12-2454]
- 190 **Seino Y**, Min KW, Niemoeller E, Takami A. Randomized, double-blind, placebo-controlled trial of the once-daily GLP-1 receptor agonist lixisenatide in Asian patients with type 2 diabetes insufficiently controlled on basal insulin with or without a sulfonylurea (GetGoal-L-Asia). *Diabetes Obes Metab* 2012; **14**: 910-917 [PMID: 22564709 DOI: 10.1111/j.1463-1326.2012.01618.x]
- 191 **Garber A**, Henry R, Ratner R, Garcia-Hernandez PA, Rodriguez-Pattzi H, Olvera-Alvarez I, Hale PM, Zdravkovic M, Bode B. Liraglutide versus glimepiride monotherapy for type 2 diabetes (LEAD-3 Mono): a randomised, 52-week, phase III, double-blind, parallel-treatment trial. *Lancet* 2009; **373**: 473-481 [PMID: 18819705 DOI: 10.1016/S0140-6736(08)61246-5]
- 192 **Buse JB**, Vilsbøll T, Thurman J, Blevins TC, Langbakke IH, Böttcher SG, Rodbard HW. Contribution of liraglutide in the fixed-ratio combination of insulin degludec and liraglutide (IDegLira). *Diabetes Care* 2014; **37**: 2926-2933 [PMID: 25114296 DOI: 10.2337/dc14-0785]
- 193 **Drucker DJ**, Buse JB, Taylor K, Kendall DM, Trautmann M, Zhuang D, Porter L. Exenatide once weekly versus twice daily for the treatment of type 2 diabetes: a randomised, open-label, non-inferiority study. *Lancet* 2008; **372**: 1240-1250 [PMID: 18782641 DOI: 10.1016/S0140-6736(08)61206-4]
- 194 **Blevins T**, Pullman J, Malloy J, Yan P, Taylor K, Schulteis C, Trautmann M, Porter L. DURATION-5: exenatide once weekly resulted in greater improvements in glycemic control compared with exenatide twice daily in patients with type 2 diabetes. *J Clin Endocrinol Metab* 2011; **96**: 1301-1310 [PMID: 21307137 DOI: 10.1210/jc.2010-2081]
- 195 **Bergental RM**, Wysham C, Macconell L, Malloy J, Walsh B, Yan P, Wilhelm K, Malone J, Porter LE. Efficacy and safety of exenatide once weekly versus sitagliptin or pioglitazone as an adjunct to metformin for treatment of type 2 diabetes (DURATION-2): a randomised trial. *Lancet* 2010; **376**: 431-439 [PMID: 20580422 DOI: 10.1016/S0140-6736(10)60590-9]
- 196 **Diamant M**, Van Gaal L, Stranks S, Northrup J, Cao D, Taylor K, Trautmann M. Once weekly exenatide compared with insulin glargine titrated to target in patients with type 2 diabetes (DURATION-3): an open-label randomised trial. *Lancet* 2010; **375**: 2234-2243 [PMID: 20609969 DOI: 10.1016/S0140-6736(10)60406-0]
- 197 **Weissman PN**, Carr MC, Ye J, Cirkel DT, Stewart M, Perry C, Pratley R. HARMONY 4: randomised clinical trial comparing once-weekly albiglutide and insulin glargine in patients with type 2 diabetes inadequately controlled with metformin with or without sulfonylurea. *Diabetologia* 2014; **57**: 2475-2484 [PMID: 25208756]



- DOI: 10.1007/s00125-014-3360-3]
- 198 **Ahrén B**, Johnson SL, Stewart M, Cirkel DT, Yang F, Perry C, Feinglos MN. HARMONY 3: 104-week randomized, double-blind, placebo- and active-controlled trial assessing the efficacy and safety of albiglutide compared with placebo, sitagliptin, and glimepiride in patients with type 2 diabetes taking metformin. *Diabetes Care* 2014; **37**: 2141-2148 [PMID: 24898304 DOI: 10.2337/dc14-0024]
  - 199 **Nauck M**, Weinstock RS, Umpierrez GE, Guerci B, Skrivanek Z, Milicevic Z. Efficacy and safety of dulaglutide versus sitagliptin after 52 weeks in type 2 diabetes in a randomized controlled trial (AWARD-5). *Diabetes Care* 2014; **37**: 2149-2158 [PMID: 24742660 DOI: 10.2337/dc13-2761]
  - 200 **Shyangdan DS**, Royle P, Clar C, Sharma P, Waugh N, Snaith A. Glucagon-like peptide analogues for type 2 diabetes mellitus. *Cochrane Database Syst Rev* 2011; **(10)**: CD006423 [PMID: 21975753 DOI: 10.1002/14651858]
  - 201 **Madsbad S**. Review of head-to-head comparisons of glucagon-like peptide-1 receptor agonists. *Diabetes Obes Metab* 2016; **18**: 317-332 [PMID: 26511102 DOI: 10.1111/dom.12596]
  - 202 **Buse JB**, Nauck M, Forst T, Sheu WH, Shenouda SK, Heilmann CR, Hoogwerf BJ, Gao A, Boardman MK, Fineman M, Porter L, Scherthaner G. Exenatide once weekly versus liraglutide once daily in patients with type 2 diabetes (DURATION-6): a randomised, open-label study. *Lancet* 2013; **381**: 117-124 [PMID: 23141817 DOI: 10.1016/S0140-6736(12)61267-7]
  - 203 **Buse JB**, Rosenstock J, Sesti G, Schmidt WE, Montanya E, Brett JH, Zychma M, Blonde L. Liraglutide once a day versus exenatide twice a day for type 2 diabetes: a 26-week randomised, parallel-group, multinational, open-label trial (LEAD-6). *Lancet* 2009; **374**: 39-47 [PMID: 19515413 DOI: 10.1016/S0140-6736(09)60659-0]
  - 204 **Pratley RE**, Nauck MA, Barnett AH, Feinglos MN, Ovalle F, Harman-Boehm I, Ye J, Scott R, Johnson S, Stewart M, Rosenstock J. Once-weekly albiglutide versus once-daily liraglutide in patients with type 2 diabetes inadequately controlled on oral drugs (HARMONY 7): a randomised, open-label, multicentre, non-inferiority phase 3 study. *Lancet Diabetes Endocrinol* 2014; **2**: 289-297 [PMID: 24703047 DOI: 10.1016/S2213-8587(13)70214-6]
  - 205 **Dungan KM**, Povedano ST, Forst T, González JG, Atisso C, Sealls W, Fahrback JL. Once-weekly dulaglutide versus once-daily liraglutide in metformin-treated patients with type 2 diabetes (AWARD-6): a randomised, open-label, phase 3, non-inferiority trial. *Lancet* 2014; **384**: 1349-1357 [PMID: 25018121 DOI: 10.1016/S0140-6736(14)60976-4]
  - 206 **Kapitza C**, Forst T, Coester HV, Poitiers F, Ruus P, Hincelin-Méry A. Pharmacodynamic characteristics of lixisenatide once daily versus liraglutide once daily in patients with type 2 diabetes insufficiently controlled on metformin. *Diabetes Obes Metab* 2013; **15**: 642-649 [PMID: 23368510 DOI: 10.1111/dom.12076]
  - 207 **Owens DR**, Matfin G, Monnier L. Basal insulin analogues in the management of diabetes mellitus: What progress have we made? *Diabetes Metab Res Rev* 2014; **30**: 104-119 [PMID: 24026961 DOI: 10.1002/dmrr.2469]
  - 208 **Horvath K**, Jeitler K, Berghold A, Ebrahim SH, Gratzner TW, Plank J, Kaiser T, Pieber TR, Siebenhofer A. Long-acting insulin analogues versus NPH insulin (human isophane insulin) for type 2 diabetes mellitus. *Cochrane Database Syst Rev* 2007; **(2)**: CD005613 [PMID: 17443605 DOI: 10.1002/14651858.CD005613.pub3]
  - 209 **Heinemann L**, Linkeschova R, Rave K, Hompesch B, Sedlak M, Heise T. Time-action profile of the long-acting insulin analog insulin glargine (HOE901) in comparison with those of NPH insulin and placebo. *Diabetes Care* 2000; **23**: 644-649 [PMID: 10834424 DOI: 10.2337/diacare.23.5.644]
  - 210 **Sanches AC**, Correr CJ, Venson R, Pontarolo R. Revisiting the efficacy of long-acting insulin analogues on adults with type 1 diabetes using mixed-treatment comparisons. *Diabetes Res Clin Pract* 2011; **94**: 333-339 [PMID: 21992870 DOI: 10.1016/j.diabres.2011.09.001]
  - 211 **Heise T**, Pieber TR. Towards peakless, reproducible and long-acting insulins. An assessment of the basal analogues based on isoglycaemic clamp studies. *Diabetes Obes Metab* 2007; **9**: 648-659 [PMID: 17645556 DOI: 10.1111/j.1463-1326.2007.00756.x]
  - 212 **Ashwell SG**, Gebbie J, Home PD. Twice-daily compared with once-daily insulin glargine in people with Type 1 diabetes using meal-time insulin aspart. *Diabet Med* 2006; **23**: 879-886 [PMID: 16911626 DOI: 10.1111/j.1464-5491.2006.01913.x]
  - 213 **Ratner RE**, Gough SC, Mathieu C, Del Prato S, Bode B, Mersebach H, Endahl L, Zinman B. Hypoglycaemia risk with insulin degludec compared with insulin glargine in type 2 and type 1 diabetes: a pre-planned meta-analysis of phase 3 trials. *Diabetes Obes Metab* 2013; **15**: 175-184 [PMID: 23130654 DOI: 10.1111/dom.12032]
  - 214 **Garber AJ**, King AB, Del Prato S, Sreenan S, Balci MK, Muñoz-Torres M, Rosenstock J, Endahl LA, Francisco AM, Hollander P. Insulin degludec, an ultra-longacting basal insulin, versus insulin glargine in basal-bolus treatment with mealtime insulin aspart in type 2 diabetes (BEGIN Basal-Bolus Type 2): a phase 3, randomised, open-label, treat-to-target non-inferiority trial. *Lancet* 2012; **379**: 1498-1507 [PMID: 22521072]
  - 215 **Hollander P**, King AB, Del Prato S, Sreenan S, Balci MK, Muñoz-Torres M, Rosenstock J, Hansen CT, Niemeyer M, Garber AJ. Insulin degludec improves long-term glycaemic control similarly to insulin glargine but with fewer hypoglycaemic episodes in patients with advanced type 2 diabetes on basal-bolus insulin therapy. *Diabetes Obes Metab* 2015; **17**: 202-206 [PMID: 25387855 DOI: 10.1111/dom.12411]
  - 216 **Zinman B**, Philis-Tsimikas A, Cariou B, Handelsman Y, Rodbard HW, Johansen T, Endahl L, Mathieu C. Insulin degludec versus insulin glargine in insulin-naïve patients with type 2 diabetes: a 1-year, randomized, treat-to-target trial (BEGIN Once Long). *Diabetes Care* 2012; **35**: 2464-2471 [PMID: 23043166]
  - 217 **Riddle MC**, Bolli GB, Ziemien M, Muehlen-Bartmer I, Bizet F, Home PD. New insulin glargine 300 units/mL versus glargine 100 units/mL in people with type 2 diabetes using basal and mealtime insulin: glucose control and hypoglycemia in a 6-month randomized controlled trial (EDITION 1). *Diabetes Care* 2014; **37**: 2755-2762 [PMID: 25078900 DOI: 10.2337/dc14-0991]
  - 218 **Yki-Järvinen H**, Bergenstal R, Ziemien M, Wardecki M, Muehlen-Bartmer I, Boelle E, Riddle MC. New insulin glargine 300 units/mL versus glargine 100 units/mL in people with type 2 diabetes using oral agents and basal insulin: glucose control and hypoglycemia in a 6-month randomized controlled trial (EDITION 2). *Diabetes Care* 2014; **37**: 3235-3243 [PMID: 25193531 DOI: 10.2337/dc14-0990]
  - 219 **Bolli GB**, Riddle MC, Bergenstal RM, Ziemien M, Sestakauskas K, Goyeau H, Home PD. New insulin glargine 300 U/ml compared with glargine 100 U/ml in insulin-naïve people with type 2 diabetes on oral glucose-lowering drugs: a randomized controlled trial (EDITION 3). *Diabetes Obes Metab* 2015; **17**: 386-394 [PMID: 25641260 DOI: 10.1111/dom.12438]
  - 220 **Ooi CP**, Loke SC. Colesevelam for type 2 diabetes mellitus. *Cochrane Database Syst Rev* 2012; **12**: CD009361 [PMID: 23235674 DOI: 10.1002/14651858.CD009361.pub2]
  - 221 **Fonseca VA**, Rosenstock J, Wang AC, Truitt KE, Jones MR. Colesevelam HCl improves glycemic control and reduces LDL cholesterol in patients with inadequately controlled type 2 diabetes on sulfonylurea-based therapy. *Diabetes Care* 2008; **31**: 1479-1484 [PMID: 18458145 DOI: 10.2337/dc08-0283]
  - 222 **Goldberg RB**, Fonseca VA, Truitt KE, Jones MR. Efficacy and safety of colesevelam in patients with type 2 diabetes mellitus and inadequate glycemic control receiving insulin-based therapy. *Arch Intern Med* 2008; **168**: 1531-1540 [PMID: 18663165 DOI: 10.1001/archinte.168.14.1531]
  - 223 Bromocriptine (Cycloset) for type 2 diabetes. *Med Lett Drugs Ther* 2010; **52**: 97-98 [PMID: 21344781]
  - 224 **Cincotta AH**, Meier AH. Bromocriptine improves glycaemic control and serum lipid profile in obese Type 2 diabetic subjects: a new approach in the treatment of diabetes. *Expert Opin Investig Drugs* 1999; **8**: 1683-1707 [PMID: 11139820]
  - 225 **Gaziano JM**, Cincotta AH, O'Connor CM, Ezrokhi M, Rutty D, Ma ZJ, Scranton RE. Randomized clinical trial of quick-release



- bromocriptine among patients with type 2 diabetes on overall safety and cardiovascular outcomes. *Diabetes Care* 2010; **33**: 1503-1508 [PMID: 20332352 DOI: 10.2337/dc09-2009]
- 226 **Kong MF**, Stubbs TA, King P, Macdonald IA, Lambourne JE, Blackshaw PE, Perkins AC, Tattersall RB. The effect of single doses of pramlintide on gastric emptying of two meals in men with IDDM. *Diabetologia* 1998; **41**: 577-583 [PMID: 9628276]
  - 227 **Heise T**, Heinemann L, Heller S, Weyer C, Wang Y, Strobel S, Kolterman O, Maggs D. Effect of pramlintide on symptom, catecholamine, and glucagon responses to hypoglycemia in healthy subjects. *Metabolism* 2004; **53**: 1227-1232 [PMID: 15334389]
  - 228 **Hollander PA**, Levy P, Fineman MS, Maggs DG, Shen LZ, Strobel SA, Weyer C, Kolterman OG. Pramlintide as an adjunct to insulin therapy improves long-term glycemic and weight control in patients with type 2 diabetes: a 1-year randomized controlled trial. *Diabetes Care* 2003; **26**: 784-790 [PMID: 12610038]
  - 229 **Riddle M**, Pencek R, Charenkavanich S, Lutz K, Wilhelm K, Porter L. Randomized comparison of pramlintide or mealtime insulin added to basal insulin treatment for patients with type 2 diabetes. *Diabetes Care* 2009; **32**: 1577-1582 [PMID: 19502544 DOI: 10.2337/dc09-0395]
  - 230 **Ceriello A**, Lush CW, Darsow T, Piconi L, Corgnani M, Nanayakkara N, Frias JP, Maggs D. Pramlintide reduced markers of oxidative stress in the postprandial period in patients with type 2 diabetes. *Diabetes Metab Res Rev* 2008; **24**: 103-108 [PMID: 17694505]
  - 231 **Wysham C**, Lush C, Zhang B, Maier H, Wilhelm K. Effect of pramlintide as an adjunct to basal insulin on markers of cardiovascular risk in patients with type 2 diabetes. *Curr Med Res Opin* 2008; **24**: 79-85 [PMID: 18031595]
  - 232 **Li Y**, Burrows NR, Gregg EW, Albright A, Geiss LS. Declining rates of hospitalization for nontraumatic lower-extremity amputation in the diabetic population aged 40 years or older: U.S., 1988-2008. *Diabetes Care* 2012; **35**: 273-277 [PMID: 22275440 DOI: 10.2337/dc11-1360]
  - 233 **Centers for Disease Control and Prevention**. Diabetes public health resource. Available from: URL: <http://www.cdc.gov/diabetes>
  - 234 **Kirkman MS**, Briscoe VJ, Clark N, Florez H, Haas LB, Halter JB, Huang ES, Korytkowski MT, Munshi MN, Odegard PS, Pratley RE, Swift CS. Diabetes in older adults: a consensus report. *J Am Geriatr Soc* 2012; **60**: 2342-2356 [PMID: 23106132 DOI: 10.1111/jgs.12035]
  - 235 **Gómez Huelgas R**, Díez-Espino J, Formiga F, Lafita Tejedor J, Rodríguez Mañas L, González-Sarmiento E, Menéndez E, Sangrós J. Treatment of type 2 diabetes in the elderly. *Med Clin (Barc)* 2013; **140**: 134.e1-134.e12 [PMID: 23199835 DOI: 10.1016/j.medcli.2012.10.003]
  - 236 **Knowler WC**, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 2002; **346**: 393-403 [PMID: 11832527 DOI: 10.1056/NEJMoa012512]
  - 237 **Wing RR**, Hamman RF, Bray GA, Delahanty L, Edelstein SL, Hill JO, Horton ES, Hoskin MA, Kriska A, Lachin J, Mayer-Davis EJ, Pi-Sunyer X, Regensteiner JG, Venditti B, Wylie-Rosett J. Achieving weight and activity goals among diabetes prevention program lifestyle participants. *Obes Res* 2004; **12**: 1426-1434 [PMID: 15483207 DOI: 10.1038/oby.2004.179]
  - 238 **Miller CK**, Edwards L, Kissling G, Sanville L. Nutrition education improves metabolic outcomes among older adults with diabetes mellitus: results from a randomized controlled trial. *Prev Med* 2002; **34**: 252-259 [PMID: 11817922 DOI: 10.1006/pmed.2001.0985]
  - 239 **Villareal DT**, Banks M, Siener C, Sinacore DR, Klein S. Physical frailty and body composition in obese elderly men and women. *Obes Res* 2004; **12**: 913-920 [PMID: 15229329 DOI: 10.1038/oby.2004.111]
  - 240 **Villareal DT**, Banks M, Sinacore DR, Siener C, Klein S. Effect of weight loss and exercise on frailty in obese older adults. *Arch Intern Med* 2006; **166**: 860-866 [PMID: 16636211 DOI: 10.1001/archinte.166.8.860]
  - 241 **Miller SL**, Wolfe RR. The danger of weight loss in the elderly. *J Nutr Health Aging* 2008; **12**: 487-491 [PMID: 18615231 DOI: 10.1007/BF02982710]
  - 242 **Shapses SA**, Riedt CS. Bone, body weight, and weight reduction: what are the concerns? *J Nutr* 2006; **136**: 1453-1456 [PMID: 16702302]
  - 243 **Vega Piñero B**. Aspectos diferenciales de la nutrición en los pacientes ancianos con diabetes. *Av Diabetol* 2010; **26**: 307-313
  - 244 **Park SW**, Goodpaster BH, Strotmeyer ES, de Rekeneire N, Harris TB, Schwartz AV, Tylavsky FA, Newman AB. Decreased muscle strength and quality in older adults with type 2 diabetes: the health, aging, and body composition study. *Diabetes* 2006; **55**: 1813-1818 [PMID: 16731847 DOI: 10.2337/db05-1183]
  - 245 **Villareal DT**, Chode S, Parimi N, Sinacore DR, Hilton T, Armamento-Villareal R, Napoli N, Qualls C, Shah K. Weight loss, exercise, or both and physical function in obese older adults. *N Engl J Med* 2011; **364**: 1218-1229 [PMID: 21449785 DOI: 10.1056/NEJMoa1008234]
  - 246 **Buman MP**, Hekler EB, Haskell WL, Pruitt L, Conway TL, Cain KL, Sallis JF, Saelens BE, Frank LD, King AC. Objective light-intensity physical activity associations with rated health in older adults. *Am J Epidemiol* 2010; **172**: 1155-1165 [PMID: 20843864 DOI: 10.1093/aje/kwq249]
  - 247 **Lipska KJ**, Bailey CJ, Inzucchi SE. Use of metformin in the setting of mild-to-moderate renal insufficiency. *Diabetes Care* 2011; **34**: 1431-1437 [PMID: 21617112 DOI: 10.2337/dc10-2361]
  - 248 **Shorr RI**, Ray WA, Daugherty JR, Griffin MR. Individual sulfonylureas and serious hypoglycemia in older people. *J Am Geriatr Soc* 1996; **44**: 751-755 [PMID: 8675920 DOI: 10.1111/j.1532-5415.1996.tb03729.x]
  - 249 **Huang ES**, Liu JY, Moffet HH, John PM, Karter AJ. Glycemic control, complications, and death in older diabetic patients: the diabetes and aging study. *Diabetes Care* 2011; **34**: 1329-1336 [PMID: 21505211 DOI: 10.2337/dc10-2377]
  - 250 **Roussel R**, Travert F, Pasquet B, Wilson PW, Smith SC, Goto S, Ravaud P, Marre M, Porath A, Bhatt DL, Steg PG. Metformin use and mortality among patients with diabetes and atherothrombosis. *Arch Intern Med* 2010; **170**: 1892-1899 [PMID: 21098347 DOI: 10.1001/archinternmed.2010.409]
  - 251 **National Institute for Health and Care Excellence**. Type 2 diabetes in adults: management: December 2015 NICE guidelines. Available from: URL: <https://www.nice.org.uk/guidance/ng28>
  - 252 **Patel A**, MacMahon S, Chalmers J, Neal B, Billot L, Woodward M, Marre M, Cooper M, Glasziou P, Grobbee D, Hamet P, Harrap S, Heller S, Liu L, Mancia G, Mogensen CE, Pan C, Poulter N, Rodgers A, Williams B, Bompont S, de Galan BE, Joshi R, Travert F. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. *N Engl J Med* 2008; **358**: 2560-2572 [PMID: 18539916 DOI: 10.1056/NEJMoa0802987]
  - 253 **The American Geriatrics Society 2015 Beers Criteria Update Expert Panel**. American Geriatrics Society 2015 Updated Beers Criteria for Potentially Inappropriate Medication Use in Older Adults. *J Am Geriatr Soc* 2015; **63**: 2227-2246 [PMID: 26446832 DOI: 10.1111/jgs.13702]
  - 254 **Shorr RI**, Ray WA, Daugherty JR, Griffin MR. Incidence and risk factors for serious hypoglycemia in older persons using insulin or sulfonylureas. *Arch Intern Med* 1997; **157**: 1681-1686 [PMID: 9250229 DOI: 10.1001/archinte.1997.00440360095010]
  - 255 **Bressler R**, Johnson DG. Oral antidiabetic drug use in the elderly. *Drugs Aging* 1996; **9**: 418-437 [PMID: 8972242 DOI: 10.2165/00002512-199609060-00005]
  - 256 **Papa G**, Fedele V, Rizzo MR, Fioravanti M, Leotta C, Solerte SB, Purrello F, Paolisso G. Safety of type 2 diabetes treatment with repaglinide compared with glibenclamide in elderly people: A randomized, open-label, two-period, cross-over trial. *Diabetes Care* 2006; **29**: 1918-1920 [PMID: 16873803 DOI: 10.2337/dc05-2495]
  - 257 **Bloomgarden Z**, Drexler A. What role will 'gliptins' play in glycemic control? *Cleve Clin J Med* 2008; **75**: 305-310 [PMID: 18491437]
  - 258 **Hsieh CJ**. Acarbose reduces the risk of pre-lunch hypoglycemia in elderly people with diabetes eating rice porridge for breakfast.

- Diabetes Res Clin Pract* 2010; **89**: e66-e68 [PMID: 20619914 DOI: 10.1016/j.diabres.2010.05.030]
- 259 **Dormandy JA**, Charbonnel B, Eckland DJ, Erdmann E, Massi-Benedetti M, Moules IK, Skene AM, Tan MH, Lefèbvre PJ, Murray GD, Standl E, Wilcox RG, Wilhelmsen L, Betteridge J, Birkeland K, Golay A, Heine RJ, Korányi L, Laakso M, Mokán M, Norkus A, Pirags V, Podar T, Scheen A, Scherbaum W, Scherthaner G, Schmitz O, Skrha J, Smith U, Taton J. Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial In macroVascular Events): a randomised controlled trial. *Lancet* 2005; **366**: 1279-1289 [PMID: 16214598 DOI: 10.1016/S0140-6736(05)67528-9]
  - 260 **Pioglitazona**: resultados de la evaluación europea sobre su posible asociación con el cáncer de vejiga [revista electrónica]. Available from: URL: [http://www.aemps.gob.es/informa/notasInformativas/medicamentosUsoHumano/seguridad/2011/NI-MUH\\_13-2011.htm](http://www.aemps.gob.es/informa/notasInformativas/medicamentosUsoHumano/seguridad/2011/NI-MUH_13-2011.htm)
  - 261 **Waugh J**, Keating GM, Plosker GL, Easthope S, Robinson DM. Pioglitazone: a review of its use in type 2 diabetes mellitus. *Drugs* 2006; **66**: 85-109 [PMID: 16398569]
  - 262 **Schweizer A**, Dejager S, Foley JE, Shao Q, Kothny W. Clinical experience with vildagliptin in the management of type 2 diabetes in a patient population  $\geq 75$  years: a pooled analysis from a database of clinical trials. *Diabetes Obes Metab* 2011; **13**: 55-64 [PMID: 21114604 DOI: 10.1111/j.1463-1326.2010.01325.x]
  - 263 **McEwen A**, Mc Kay GA, Fisher M. Drugs for diabetes. Part 8: SGLT2 inhibitors. *Br J Cardiol* 2012; **19**: 26-29 [DOI: 10.5837/bjc.2012.005]
  - 264 **Demaris KM**, White JR. Dapagliflozin, an SGLT2 inhibitor for the treatment of type 2 diabetes. *Drugs Today (Barc)* 2013; **49**: 289-301 [PMID: 23724409 DOI: 10.1358/dot.2013.49.5.1964714]
  - 265 **Elmore LK**, Baggett S, Kyle JA, Skelley JW. A review of the efficacy and safety of canagliflozin in elderly patients with type 2 diabetes. *Consult Pharm* 2014; **29**: 335-346 [PMID: 24849690 DOI: 10.4140/TCP.n.2014.335]
  - 266 **Ferrannini E**, Solini A. SGLT2 inhibition in diabetes mellitus: rationale and clinical prospects. *Nat Rev Endocrinol* 2012; **8**: 495-502 [PMID: 22310849 DOI: 10.1038/nrendo.2011.243]
  - 267 **Herman WH**, Ilag LL, Johnson SL, Martin CL, Sinding J, Al Harthi A, Plunkett CD, LaPorte FB, Burke R, Brown MB, Halter JB, Raskin P. A clinical trial of continuous subcutaneous insulin infusion versus multiple daily injections in older adults with type 2 diabetes. *Diabetes Care* 2005; **28**: 1568-1573 [PMID: 15983302 DOI: 10.2337/diacare.28.7.1568]
  - 268 **Lee P**, Chang A, Blaum C, Vlainic A, Gao L, Halter J. Comparison of safety and efficacy of insulin glargine and neutral protamine hagedorn insulin in older adults with type 2 diabetes mellitus: results from a pooled analysis. *J Am Geriatr Soc* 2012; **60**: 51-59 [PMID: 22239291 DOI: 10.1111/j.1532-5415.2011.03773.x]
  - 269 **Home PD**, Fritsche A, Schinzel S, Massi-Benedetti M. Meta-analysis of individual patient data to assess the risk of hypoglycaemia in people with type 2 diabetes using NPH insulin or insulin glargine. *Diabetes Obes Metab* 2010; **12**: 772-779 [PMID: 20649629 DOI: 10.1111/j.1463-1326.2010.01232.x]
  - 270 **Siebenhofer A**, Plank J, Berghold A, Narath M, Gfrerer R, Pieber TR. Short acting insulin analogues versus regular human insulin in patients with diabetes mellitus. *Cochrane Database Syst Rev* 2004; **(2)**: CD003287 [PMID: 15106199 DOI: 10.1002/14651858.CD003287.pub2]
  - 271 **International Diabetes Federation**. Global Guidelines for managing older people with type 2 diabetes (Glucose control, management and targets. 2015: 35). Available from: URL: <http://www.idf.org>
  - 272 **Garg R**, Williams ME. Diabetes management in the kidney patient. *Med Clin North Am* 2013; **97**: 135-156 [PMID: 23290735 DOI: 10.1016/j.mcna.2012.11.001]
  - 273 **Nogueira C**, Souto SB, Vinha E, Braga DC, Carvalho D. Oral glucose lowering drugs in type 2 diabetic patients with chronic kidney disease. *Hormones (Athens)* 2013; **12**: 483-494 [PMID: 24457396 DOI: 10.14310/horm.2002.1436]
  - 274 **Alsahli M**, Gerich JE. Hypoglycemia in Patients with Diabetes and Renal Disease. *J Clin Med* 2015; **4**: 948-964 [PMID: 26239457 DOI: 10.3390/jcm4050948]
  - 275 **Torffvit O**, Lindqvist A, Agardh CD, Pahlm O. The association between diabetic nephropathy and autonomic nerve function in type 1 diabetic patients. *Scand J Clin Lab Invest* 1997; **57**: 183-191 [PMID: 9200278 DOI: 10.1080/00365519709056387]
  - 276 **Bonds DE**, Miller ME, Bergenstal RM, Buse JB, Byington RP, Cutler JA, Dudl RJ, Ismail-Beigi F, Kimel AR, Hoogwerf B, Horowitz KR, Savage PJ, Seaquist ER, Simmons DL, Sivitz WI, Speril-Hillen JM, Sweeney ME. The association between symptomatic, severe hypoglycaemia and mortality in type 2 diabetes: retrospective epidemiological analysis of the ACCORD study. *BMJ* 2010; **340**: b4909 [PMID: 20061358 DOI: 10.1136/bmj.b4909]
  - 277 **Ceriello A**, Novials A, Ortega E, La Sala L, Pujadas G, Testa R, Bonfigli AR, Esposito K, Giugliano D. Evidence that hyperglycemia after recovery from hypoglycemia worsens endothelial function and increases oxidative stress and inflammation in healthy control subjects and subjects with type 1 diabetes. *Diabetes* 2012; **61**: 2993-2997 [PMID: 22891214 DOI: 10.2337/db12-0224]
  - 278 **Zoungas S**, Patel A, Chalmers J, de Galan BE, Li Q, Billot L, Woodward M, Ninomiya T, Neal B, MacMahon S, Grobbee DE, Kengne AP, Marre M, Heller S. Severe hypoglycemia and risks of vascular events and death. *N Engl J Med* 2010; **363**: 1410-1418 [PMID: 20925543 DOI: 10.1056/NEJMoa1003795]
  - 279 **National Kidney Foundation**. KDOQI Clinical Practice Guideline for Diabetes and CKD: 2012 Update. *Am J Kidney Dis* 2012; **60**: 850-886 [PMID: 23067652 DOI: 10.1053/j.ajkd.2012.07.005]
  - 280 **Sambol NC**, Chiang J, Lin ET, Goodman AM, Liu CY, Benet LZ, Cogan MG. Kidney function and age are both predictors of pharmacokinetics of metformin. *J Clin Pharmacol* 1995; **35**: 1094-1102 [PMID: 8626883 DOI: 10.1002/j.1552-4604.1995.tb04033.x]
  - 281 **Frid A**, Serner GN, Löndahl M, Wiklander C, Cato A, Vinge E, Andersson A. Novel assay of metformin levels in patients with type 2 diabetes and varying levels of renal function: clinical recommendations. *Diabetes Care* 2010; **33**: 1291-1293 [PMID: 20215446 DOI: 10.2337/dc09-1284]
  - 282 **Liu F**, Lu JX, Tang JL, Li L, Lu HJ, Hou XH, Jia WP, Xiang KS. Relationship of plasma creatinine and lactic acid in type 2 diabetic patients without renal dysfunction. *Chin Med J (Engl)* 2009; **122**: 2547-2553 [PMID: 19951568]
  - 283 **Lin YC**, Lin LY, Wang HF, Lin HD. Fasting plasma lactate concentrations in ambulatory elderly patients with type 2 diabetes receiving metformin therapy: a retrospective cross-sectional study. *J Chin Med Assoc* 2010; **73**: 617-622 [PMID: 21145508 DOI: 10.1016/S1726-4901(10)70135-0]
  - 284 **Lim VC**, Sum CF, Chan ES, Yeoh LY, Lee YM, Lim SC. Lactate levels in Asian patients with type 2 diabetes mellitus on metformin and its association with dose of metformin and renal function. *Int J Clin Pract* 2007; **61**: 1829-1833 [PMID: 17887995 DOI: 10.1111/j.1742-1241.2007.01487.x]
  - 285 **Duong JK**, Roberts DM, Furlong TJ, Kumar SS, Greenfield JR, Kirkpatrick CM, Graham GG, Williams KM, Day RO. Metformin therapy in patients with chronic kidney disease. *Diabetes Obes Metab* 2012; **14**: 963-965 [PMID: 22564555 DOI: 10.1111/j.1463-1326.2012.01617.x]
  - 286 **Duong JK**, Kumar SS, Kirkpatrick CM, Greenup LC, Arora M, Lee TC, Timmins P, Graham GG, Furlong TJ, Greenfield JR, Williams KM, Day RO. Population pharmacokinetics of metformin in healthy subjects and patients with type 2 diabetes mellitus: simulation of doses according to renal function. *Clin Pharmacokinet* 2013; **52**: 373-384 [PMID: 23475568 DOI: 10.1007/s40262-013-0046-9]
  - 287 **Stacul F**, van der Molen AJ, Reimer P, Webb JA, Thomsen HS, Morcos SK, Almén T, Aspelin P, Bellin MF, Clement O, Heinz-Peer G. Contrast induced nephropathy: updated ESUR Contrast Media Safety Committee guidelines. *Eur Radiol* 2011; **21**: 2527-2541 [PMID: 21866433 DOI: 10.1007/s00330-011-2225-0]
  - 288 **Schejter YD**, Turvall E, Ackerman Z. Characteristics of patients with

- sulphonurea-induced hypoglycemia. *J Am Med Dir Assoc* 2012; **13**: 234-238 [PMID: 21450199 DOI: 10.1016/j.jamda.2010.07.014]
- 289 **Holstein A**, Plaschke A, Hammer C, Egberts EH. Characteristics and time course of severe glimepiride- versus glibenclamide-induced hypoglycaemia. *Eur J Clin Pharmacol* 2003; **59**: 91-97 [PMID: 12698302 DOI: 10.1007/s00228-003-0592-4]
- 290 **Rosenkranz B**, Profozic V, Metelko Z, Mrzljak V, Lange C, Malerczyk V. Pharmacokinetics and safety of glimepiride at clinically effective doses in diabetic patients with renal impairment. *Diabetologia* 1996; **39**: 1617-1624 [PMID: 8960852 DOI: 10.1007/s001250050624]
- 291 **Canadian Diabetes Association Clinical Practice Guidelines Expert Committee**. Canadian Diabetes Association. 2013 Clinical Practice Guidelines for the Prevention and Management of Diabetes in Canada. Chronic kidney disease in diabetes. *Can J Diabetes* 2013; **37** (Suppl 1): S129-S136 [DOI: 10.1016/j.cjcd.2013.01.037]
- 292 **Zanchi A**, Lehmann R, Philippe J. Antidiabetic drugs and kidney disease--recommendations of the Swiss Society for Endocrinology and Diabetology. *Swiss Med Wkly* 2012; **142**: w13629 [PMID: 22987488 DOI: 10.4414/SMW.2012.13629]
- 293 **Scheen AJ**. Pharmacokinetic considerations for the treatment of diabetes in patients with chronic kidney disease. *Expert Opin Drug Metab Toxicol* 2013; **9**: 529-550 [PMID: 23461781 DOI: 10.1517/17425255.2013.777428]
- 294 **Sarkar A**, Tiwari A, Bhasin PS, Mitra M. Pharmacological and Pharmaceutical Profile of Gliclazide: A Review. *J App Pharmaceut Sci* 2011; **1**: 11-19
- 295 **Schumacher S**, Abbasi I, Weise D, Hatorp V, Sattler K, Sieber J, Hasslacher C. Single- and multiple-dose pharmacokinetics of repaglinide in patients with type 2 diabetes and renal impairment. *Eur J Clin Pharmacol* 2001; **57**: 147-152 [PMID: 11417447 DOI: 10.1016/S0168-8227(00)81702-7]
- 296 **Hatorp V**. Clinical pharmacokinetics and pharmacodynamics of repaglinide. *Clin Pharmacokinet* 2002; **41**: 471-483 [PMID: 12083976 DOI: 10.2165/00003088-200241070-00002]
- 297 **McLeod JF**. Clinical pharmacokinetics of nateglinide: a rapidly-absorbed, short-acting insulinotropic agent. *Clin Pharmacokinet* 2004; **43**: 97-120 [PMID: 14748619 DOI: 10.2165/00003088-200443020-00003]
- 298 **Inoue T**, Shibahara N, Miyagawa K, Itahana R, Izumi M, Nakanishi T, Takamitsu Y. Pharmacokinetics of nateglinide and its metabolites in subjects with type 2 diabetes mellitus and renal failure. *Clin Nephrol* 2003; **60**: 90-95 [PMID: 12940610 DOI: 10.5414/CNP60090]
- 299 **Scott LJ**, Spencer CM. Miglitrol: a review of its therapeutic potential in type 2 diabetes mellitus. *Drugs* 2000; **59**: 521-549 [PMID: 10776834 DOI: 10.2165/00003495-200059030-00012]
- 300 **Abe M**, Kikuchi F, Kaizu K, Matsumoto K. Combination therapy of pioglitazone with voglibose improves glycemic control safely and rapidly in Japanese type 2-diabetic patients on hemodialysis. *Clin Nephrol* 2007; **68**: 287-294 [PMID: 18044260]
- 301 **Abe M**, Okada K, Maruyama T, Maruyama N, Matsumoto K. Combination therapy with mitglinide and voglibose improves glycemic control in type 2 diabetic patients on hemodialysis. *Expert Opin Pharmacother* 2010; **11**: 169-176 [PMID: 20025554 DOI: 10.1517/14656560903530683]
- 302 **Snyder RW**, Berns JS. Use of insulin and oral hypoglycemic medications in patients with diabetes mellitus and advanced kidney disease. *Semin Dial* 2004; **17**: 365-370 [PMID: 15461745 DOI: 10.1111/j.0894-0959.2004.17346.x]
- 303 **Budde K**, Neumayer HH, Fritsche L, Sulowicz W, Stompör T, Eckland D. The pharmacokinetics of pioglitazone in patients with impaired renal function. *Br J Clin Pharmacol* 2003; **55**: 368-374 [PMID: 12680885 DOI: 10.1046/j.1365-2125.2003.01785.x]
- 304 **Cheng D**, Fei Y, Liu Y, Li J, Chen Y, Wang X, Wang N. Efficacy and safety of dipeptidyl peptidase-4 inhibitors in type 2 diabetes mellitus patients with moderate to severe renal impairment: a systematic review and meta-analysis. *PLoS One* 2014; **9**: e111543 [PMID: 25360775 DOI: 10.1371/journal.pone.0111543]
- 305 **Mikhail N**. Use of dipeptidyl peptidase-4 inhibitors for the treatment of patients with type 2 diabetes mellitus and chronic kidney disease. *Postgrad Med* 2012; **124**: 138-144 [PMID: 22913902 DOI: 10.3810/pgm.2012.07.2575]
- 306 **Nowicki M**, Rychlik I, Haller H, Warren M, Suchower L, Gause-Nilsson I, Schützer KM. Long-term treatment with the dipeptidyl peptidase-4 inhibitor saxagliptin in patients with type 2 diabetes mellitus and renal impairment: a randomised controlled 52-week efficacy and safety study. *Int J Clin Pract* 2011; **65**: 1230-1239 [PMID: 21977965 DOI: 10.1111/j.1742-1241.2011.02812.x]
- 307 **Chan JC**, Scott R, Arjona Ferreira JC, Sheng D, Gonzalez E, Davies MJ, Stein PP, Kaufman KD, Amatruda JM, Williams-Herman D. Safety and efficacy of sitagliptin in patients with type 2 diabetes and chronic renal insufficiency. *Diabetes Obes Metab* 2008; **10**: 545-555 [PMID: 18518892 DOI: 10.1111/j.1463-1326.2008.00914.x]
- 308 **Lukashevich V**, Schweizer A, Shao Q, Groop PH, Kothny W. Safety and efficacy of vildagliptin versus placebo in patients with type 2 diabetes and moderate or severe renal impairment: a prospective 24-week randomized placebo-controlled trial. *Diabetes Obes Metab* 2011; **13**: 947-954 [PMID: 21733061 DOI: 10.1111/j.1463-1326.2011.01467.x]
- 309 **Kothny W**, Shao Q, Groop PH, Lukashevich V. One-year safety, tolerability and efficacy of vildagliptin in patients with type 2 diabetes and moderate or severe renal impairment. *Diabetes Obes Metab* 2012; **14**: 1032-1039 [PMID: 22690943 DOI: 10.1111/j.1463-1326.2012.01634.x]
- 310 **Scheen AJ**. Linagliptin for the treatment of type 2 diabetes (pharmacokinetic evaluation). *Expert Opin Drug Metab Toxicol* 2011; **7**: 1561-1576 [PMID: 22022857 DOI: 10.1517/17425255.2011.628986]
- 311 **McGill JB**, Sloan L, Newman J, Patel S, Sauce C, von Eynatten M, Woerle HJ. Long-term efficacy and safety of linagliptin in patients with type 2 diabetes and severe renal impairment: a 1-year, randomized, double-blind, placebo-controlled study. *Diabetes Care* 2013; **36**: 237-244 [PMID: 23033241 DOI: 10.2337/dc12-0706]
- 312 **Herman GA**, Stevens C, Van Dyck K, Bergman A, Yi B, De Smet M, Snyder K, Hilliard D, Tanen M, Tanaka W, Wang AQ, Zeng W, Musson D, Winchell G, Davies MJ, Rameel S, Gottesdiener KM, Wagner JA. Pharmacokinetics and pharmacodynamics of sitagliptin, an inhibitor of dipeptidyl peptidase IV, in healthy subjects: results from two randomized, double-blind, placebo-controlled studies with single oral doses. *Clin Pharmacol Ther* 2005; **78**: 675-688 [PMID: 16338283 DOI: 10.1016/j.clpt.2005.09.002]
- 313 **He YL**. Clinical pharmacokinetics and pharmacodynamics of vildagliptin. *Clin Pharmacokinet* 2012; **51**: 147-162 [PMID: 22339447 DOI: 10.2165/11598080-000000000-00000]
- 314 **Fura A**, Khanna A, Vyas V, Koplowitz B, Chang SY, Caporuscio C, Boulton DW, Christopher LJ, Chadwick KD, Hamann LG, Humphreys WG, Kirby M. Pharmacokinetics of the dipeptidyl peptidase 4 inhibitor saxagliptin in rats, dogs, and monkeys and clinical projections. *Drug Metab Dispos* 2009; **37**: 1164-1171 [PMID: 19251818 DOI: 10.1124/dmd.108.026088]
- 315 **Graefe-Mody U**, Retlich S, Friedrich C. Clinical pharmacokinetics and pharmacodynamics of linagliptin. *Clin Pharmacokinet* 2012; **51**: 411-427 [PMID: 22568694 DOI: 10.2165/11630900-000000000-00000]
- 316 **Graefe-Mody U**, Friedrich C, Port A, Ring A, Retlich S, Heise T, Halabi A, Woerle HJ. Effect of renal impairment on the pharmacokinetics of the dipeptidyl peptidase-4 inhibitor linagliptin(\*). *Diabetes Obes Metab* 2011; **13**: 939-946 [PMID: 21672124 DOI: 10.1111/j.1463-1326.2011.01458.x]
- 317 **Barnett AH**. Linagliptin: a novel dipeptidyl peptidase 4 inhibitor with a unique place in therapy. *Adv Ther* 2011; **28**: 447-459 [PMID: 21603986 DOI: 10.1007/s12325-011-0028-y]
- 318 **Deacon CF**. Dipeptidyl peptidase-4 inhibitors in the treatment of type 2 diabetes: a comparative review. *Diabetes Obes Metab* 2011; **13**: 7-18 [PMID: 21114598 DOI: 10.1111/j.1463-1326.2010.01306.x]
- 319 **Yale JF**, Bakris G, Cariou B, Yue D, David-Neto E, Xi L, Figueroa K, Wajs E, Usiskin K, Meininger G. Efficacy and safety of



- canagliflozin in subjects with type 2 diabetes and chronic kidney disease. *Diabetes Obes Metab* 2013; **15**: 463-473 [PMID: 23464594 DOI: 10.1111/dom.12090]
- 320 **Kohan DE**, Fioretto P, Tang W, List JF. Long-term study of patients with type 2 diabetes and moderate renal impairment shows that dapagliflozin reduces weight and blood pressure but does not improve glycemic control. *Kidney Int* 2014; **85**: 962-971 [PMID: 24067431 DOI: 10.1038/ki.2013.356]
- 321 **Barnett AH**, Mithal A, Manassie J, Jones R, Rattunde H, Woerle HJ, Broedl UC. Efficacy and safety of empagliflozin added to existing antidiabetes treatment in patients with type 2 diabetes and chronic kidney disease: a randomised, double-blind, placebo-controlled trial. *Lancet Diabetes Endocrinol* 2014; **2**: 369-384 [PMID: 24795251 DOI: 10.1016/S2213-8587(13)70208-0]
- 322 **Ferrannini E**, Veltkamp SA, Smulders RA, Kadokura T. Renal glucose handling: impact of chronic kidney disease and sodium-glucose cotransporter 2 inhibition in patients with type 2 diabetes. *Diabetes Care* 2013; **36**: 1260-1265 [PMID: 23359360 DOI: 10.2337/dc12-1503]
- 323 **Weise WJ**, Sivanandy MS, Block CA, Comi RJ. Exenatide-associated ischemic renal failure. *Diabetes Care* 2009; **32**: e22-e23 [PMID: 19171732 DOI: 10.2337/dc08-1309]
- 324 **Copley K**, McCowen K, Hiles R, Nielsen LL, Young A, Parkes DG. Investigation of exenatide elimination and its in vivo and in vitro degradation. *Curr Drug Metab* 2006; **7**: 367-374 [PMID: 16724926 DOI: 10.2174/138920006776873490]
- 325 **Linnebjerg H**, Kothare PA, Park S, Mace K, Reddy S, Mitchell M, Lins R. Effect of renal impairment on the pharmacokinetics of exenatide. *Br J Clin Pharmacol* 2007; **64**: 568-569 [DOI: 10.1111/j.1365-2125.2007.02890.x]
- 326 **Jacobsen LV**, Hindsberger C, Robson R, Zdravkovic M. Effect of renal impairment on the pharmacokinetics of the GLP-1 analogue liraglutide. *Br J Clin Pharmacol* 2009; **68**: 898-905 [PMID: 20002084 DOI: 10.1111/j.1365-2125.2009.03536.x]
- 327 **Leiter LA**, Gómez-Huelgas R, Ambos A, Arteaga JM, Marchesini G, Nikonova E, Shestakova M, Stager W, Tambascia M, Hanefeld M. Lixisenatide is Effective and Well Tolerated in Patients with Type 2 Diabetes Mellitus and Renal Impairment. *Can J Diabetes* 2014; **38** (Suppl 5): S10-S11 [DOI: 10.1016/j.jcjd.2014.07.023]
- 328 **Young MA**, Wald JA, Matthews JE, Yang F, Reinhardt RR. Effect of renal impairment on the pharmacokinetics, efficacy, and safety of albiglutide. *Postgrad Med* 2014; **126**: 35-46 [PMID: 24918790 DOI: 10.3810/pgm.2014.05.2754]
- 329 **Kuritzky L**, Umpierrez G, Ekoé JM, Mancillas-Adame L, Landó LF. Safety and efficacy of dulaglutide, a once weekly GLP-1 receptor agonist, for the management of type 2 diabetes. *Postgrad Med* 2014; **126**: 60-72 [PMID: 25414935 DOI: 10.3810/pgm.2014.10.2821]
- 330 **Rabkin R**, Ryan MP, Duckworth WC. The renal metabolism of insulin. *Diabetologia* 1984; **27**: 351-357 [PMID: 6389240 DOI: 10.1007/BF00304849]
- 331 **Mühlhauser I**, Toth G, Sawicki PT, Berger M. Severe hypoglycemia in type I diabetic patients with impaired kidney function. *Diabetes Care* 1991; **14**: 344-346 [PMID: 2060440 DOI: 10.2337/diacare.14.4.344]
- 332 **Charpentier G**, Riveline JP, Varroud-Vial M. Management of drugs affecting blood glucose in diabetic patients with renal failure. *Diabetes Metab* 2000; **26** Suppl 4: 73-85 [PMID: 10922977]
- 333 **Reilly JB**, Berns JS. Selection and dosing of medications for management of diabetes in patients with advanced kidney disease. *Semin Dial* 2010; **23**: 163-168 [PMID: 20210915 DOI: 10.1111/j.1525-139X.2010.00703.x]
- 334 **Morello CM**. Pharmacokinetics and pharmacodynamics of insulin analogs in special populations with type 2 diabetes mellitus. *Int J Gen Med* 2011; **4**: 827-835 [PMID: 22267935 DOI: 10.2147/IJGM.S26889]
- 335 **Baldwin D**, Zander J, Munoz C, Raghu P, DeLange-Hudec S, Lee H, Emanuele MA, Glossop V, Smallwood K, Molitch M. A randomized trial of two weight-based doses of insulin glargine and glulisine in hospitalized subjects with type 2 diabetes and renal insufficiency. *Diabetes Care* 2012; **35**: 1970-1974 [PMID: 22699288 DOI: 10.2337/dc12-0578]
- 336 **Ersoy A**, Ersoy C, Altinay T. Insulin analogue usage in a haemodialysis patient with type 2 diabetes mellitus. *Nephrol Dial Transplant* 2006; **21**: 553-554 [PMID: 16221693 DOI: 10.1093/ndt/gfi205]
- 337 **Gómez-Huelgas R**, Martínez-Castelao A, Artola S, Górriz JL, Menéndez E. Treatment of type 2 diabetes mellitus in patients with chronic kidney disease. Grupo de Trabajo para el Documento de Consenso sobre el tratamiento de la diabetes tipo 2 en el paciente con enfermedad renal crónica. *Med Clin (Barc)* 2014; **142**: 85.e1-85.10 [PMID: 24268912 DOI: 10.1016/j.medcli.2013.10.011]
- 338 **Frühbeck G**. Bariatric and metabolic surgery: a shift in eligibility and success criteria. *Nat Rev Endocrinol* 2015; **11**: 465-477 [PMID: 26055046 DOI: 10.1038/nrendo.2015.84]
- 339 **Singh AK**, Singh R, Kota SK. Bariatric surgery and diabetes remission: Who would have thought it? *Indian J Endocrinol Metab* 2015; **19**: 563-576 [PMID: 26425464 DOI: 10.4103/2230-8210.163113]
- 340 **Solayman M**, Ali Y, Alam F, Islam MA, Alam N, Khalil MI, Gan SH. Polyphenols: Potential Future Arsenals in the Treatment of Diabetes. *Curr Pharm Des* 2016; **22**: 549-565 [PMID: 26601968 DOI: 10.2174/1381612822666151125001111]
- 341 **Yu J**, Zhang Y, Ye Y, DiSanto R, Sun W, Ranson D, Ligler FS, Buse JB, Gu Z. Microneedle-array patches loaded with hypoxia-sensitive vesicles provide fast glucose-responsive insulin delivery. *Proc Natl Acad Sci USA* 2015; **112**: 8260-8265 [PMID: 26100900 DOI: 10.1073/pnas.1505405112]
- 342 **Finan B**, Ma T, Ottaway N, Müller TD, Habegger KM, Heppner KM, Kirchner H, Holland J, Hembree J, Raver C, Lockie SH, Smiley DL, Gelfanov V, Yang B, Hofmann S, Bruemmer D, Drucker DJ, Pfluger PT, Perez-Tilve D, Gidda J, Vignati L, Zhang L, Hauptman JB, Lau M, Brecheisen M, Uhles S, Riboulet W, Hainaut E, Sebokova E, Conde-Knape K, Konkar A, DiMarchi RD, Tschöp MH. Unimolecular dual incretins maximize metabolic benefits in rodents, monkeys, and humans. *Sci Transl Med* 2013; **5**: 209ra151 [PMID: 24174327 DOI: 10.1126/scitranslmed.3007218]
- 343 **Deryabina MA**, Dagaard JR, Knudsen CB, Shelton PT, Fog JU, Jessen L, Noerregaard P. Pharmacokinetics and pharmacodynamics of GLP-1-GIP receptor dual agonist peptides: from once-daily to once-weekly. Boston: American Diabetes Association (ADA), 2015: June 05-09
- 344 **Hjorth SA**, Adelhorst K, Pedersen BB, Kirk O, Schwartz TW. Glucagon and glucagon-like peptide 1: selective receptor recognition via distinct peptide epitopes. *J Biol Chem* 1994; **269**: 30121-30124 [PMID: 7527026]
- 345 **Orskov C**. Glucagon-like peptide-1, a new hormone of the entero-insular axis. *Diabetologia* 1992; **35**: 701-711 [PMID: 1324859 DOI: 10.1007/BF00429088]
- 346 **Parlevliet ET**, Heijboer AC, Schröder-van der Elst JP, Havekes LM, Romijn JA, Pijl H, Corssmit EP. Oxyntomodulin ameliorates glucose intolerance in mice fed a high-fat diet. *Am J Physiol Endocrinol Metab* 2008; **294**: E142-E147 [PMID: 17971509 DOI: 10.1152/ajpendo.00576.2007]
- 347 **Dakin CL**, Gunn I, Small CJ, Edwards CM, Hay DL, Smith DM, Ghatei MA, Bloom SR. Oxyntomodulin inhibits food intake in the rat. *Endocrinology* 2001; **142**: 4244-4250 [PMID: 11564680]
- 348 **Baggio LL**, Huang Q, Brown TJ, Drucker DJ. Oxyntomodulin and glucagon-like peptide-1 differentially regulate murine food intake and energy expenditure. *Gastroenterology* 2004; **127**: 546-558 [PMID: 15300587 DOI: 10.1053/j.gastro.2004.04.063]
- 349 **Wynne K**, Park AJ, Small CJ, Patterson M, Ellis SM, Murphy KG, Wren AM, Frost GS, Meeran K, Ghatei MA, Bloom SR. Subcutaneous oxyntomodulin reduces body weight in overweight and obese subjects: a double-blind, randomized, controlled trial. *Diabetes* 2005; **54**: 2390-2395 [PMID: 16046306 DOI: 10.2337/diabetes.54.8.2390]
- 350 **Wynne K**, Park AJ, Small CJ, Meeran K, Ghatei MA, Frost GS, Bloom SR. Oxyntomodulin increases energy expenditure in addition to decreasing energy intake in overweight and obese humans: a randomised controlled trial. *Int J Obes (Lond)* 2006; **30**: 1729-1736



- [PMID: 16619056 DOI: 10.1038/sj.jco.0803344]
- 351 **Day JW**, Ottaway N, Patterson JT, Gelfanov V, Smiley D, Gidda J, Findeisen H, Bruemmer D, Drucker DJ, Chaudhary N, Holland J, Hembree J, Abplanalp W, Grant E, Ruehl J, Wilson H, Kirchner H, Lockie SH, Hofmann S, Woods SC, Nogueiras R, Pfluger PT, Perez-Tilve D, DiMarchi R, Tschöp MH. A new glucagon and GLP-1 co-agonist eliminates obesity in rodents. *Nat Chem Biol* 2009; **5**: 749-757 [PMID: 19597507 DOI: 10.1038/nchembio.209]
  - 352 **Pocai A**, Carrington PE, Adams JR, Wright M, Eiermann G, Zhu L, Du X, Petrov A, Lassman ME, Jiang G, Liu F, Miller C, Tota LM, Zhou G, Zhang X, Sountis MM, Santoprete A, Capito E, Chicchi GG, Thornberry N, Bianchi E, Pessi A, Marsh DJ, SinhaRoy R. Glucagon-like peptide 1/glucagon receptor dual agonism reverses obesity in mice. *Diabetes* 2009; **58**: 2258-2266 [PMID: 19602537 DOI: 10.2337/db09-0278]
  - 353 **Pan CQ**, Buxton JM, Yung SL, Tom I, Yang L, Chen H, MacDougall M, Bell A, Claus TH, Clairmont KB, Whelan JP. Design of a long acting peptide functioning as both a glucagon-like peptide-1 receptor agonist and a glucagon receptor antagonist. *J Biol Chem* 2006; **281**: 12506-12515 [PMID: 16505481 DOI: 10.1074/jbc.M600127200]
  - 354 **Claus TH**, Pan CQ, Buxton JM, Yang L, Reynolds JC, Barucci N, Burns M, Ortiz AA, Rocznik S, Livingston JN, Clairmont KB, Whelan JP. Dual-acting peptide with prolonged glucagon-like peptide-1 receptor agonist and glucagon receptor antagonist activity for the treatment of type 2 diabetes. *J Endocrinol* 2007; **192**: 371-380 [PMID: 17283237 DOI: 10.1677/JOE-06-0018]
  - 355 **Arnolds S**, Dellweg S, Clair J, Dain MP, Nauck MA, Rave K, Kapitza C. Further improvement in postprandial glucose control with addition of exenatide or sitagliptin to combination therapy with insulin glargine and metformin: a proof-of-concept study. *Diabetes Care* 2010; **33**: 1509-1515 [PMID: 20357372 DOI: 10.2337/dc09-2191]
  - 356 **Buse JB**, Bergenstal RM, Glass LC, Heilmann CR, Lewis MS, Kwan AY, Hoogwerf BJ, Rosenstock J. Use of twice-daily exenatide in Basal insulin-treated patients with type 2 diabetes: a randomized, controlled trial. *Ann Intern Med* 2011; **154**: 103-112 [PMID: 21138825 DOI: 10.7326/0003-4819-154-2-201101180-00300]
  - 357 **Rosenstock J**, Ahrén B, Chow F. Once-weekly GLP-1 receptor agonist albiglutide vs titrated prandial lispro added on to titrated basal glargine in type 2 diabetes (T2D) uncontrolled on glargine plus oral agents: similar glycemic control with weight loss and less hypoglycemia. *Diabetes* 2012; **61** (Suppl 1): A15
  - 358 **Yoon NM**, Cavaghan MK, Brunelle RL, Roach P. Exenatide added to insulin therapy: a retrospective review of clinical practice over two years in an academic endocrinology outpatient setting. *Clin Ther* 2009; **31**: 1511-1523 [PMID: 19695400 DOI: 10.1016/j.clinthera.2009.07.021]
  - 359 **Rodbard HW**, Buse JB, Woo V, Vilsbøll T, Langbakke IH, Kvist K, Gough SC. Benefits of combination of insulin degludec and liraglutide are independent of baseline glycated haemoglobin level and duration of type 2 diabetes. *Diabetes Obes Metab* 2016; **18**: 40-48 [PMID: 26343931 DOI: 10.1111/dom.12574]
  - 360 Lixiland Clinical Development Program: Assessing a fixed-ratio combination of insulin glargine (100 units/ml) and Lixisenatide. Sanofi Diabetes at 51st Annual Meeting, September 14-18, 2015, Stockholm. Available from: URL: [http://www.drugs.com/nda/lyxumia\\_150914.html](http://www.drugs.com/nda/lyxumia_150914.html)
  - 361 **Overton HA**, Babbs AJ, Doel SM, Fyfe MC, Gardner LS, Griffin G, Jackson HC, Procter MJ, Rasamison CM, Tang-Christensen M, Widdowson PS, Williams GM, Reynet C. Deorphanization of a G protein-coupled receptor for oleoylethanolamide and its use in the discovery of small-molecule hypophagic agents. *Cell Metab* 2006; **3**: 167-175 [PMID: 16517404 DOI: 10.1016/j.cmet.2006.02.004]
  - 362 **Ning Y**, O'Neill K, Lan H, Pang L, Shan LX, Hawes BE, Hedrick JA. Endogenous and synthetic agonists of GPR119 differ in signalling pathways and their effects on insulin secretion in MIN6c4 insulinoma cells. *Br J Pharmacol* 2008; **155**: 1056-1065 [PMID: 18724386 DOI: 10.1038/bjp.2008.337]
  - 363 **Swaminath G**. Fatty acid binding receptors and their physiological role in type 2 diabetes. *Arch Pharm* (Weinheim) 2008; **341**: 753-761 [PMID: 19009545 DOI: 10.1002/ardp.200800096]
  - 364 **Lan H**, Vassileva G, Corona A, Liu L, Baker H, Golovko A, Abbondanzo SJ, Hu W, Yang S, Ning Y, Del Vecchio RA, Poulet F, Lavery M, Gustafson EL, Hedrick JA, Kowalski TJ. GPR119 is required for physiological regulation of glucagon-like peptide-1 secretion but not for metabolic homeostasis. *J Endocrinol* 2009; **201**: 219-230 [PMID: 19282326 DOI: 10.1677/JOE-08-0453]
  - 365 **Overton HA**, Fyfe MC, Reynet C. GPR119, a novel G protein-coupled receptor target for the treatment of type 2 diabetes and obesity. *Br J Pharmacol* 2008; **153** Suppl 1: S76-S81 [PMID: 18037923 DOI: 10.1038/sj.bjp.0707529]
  - 366 **Dhayal S**, Morgan NG. The significance of GPR119 agonists as a future treatment for type 2 diabetes. *Drug News Perspect* 2010; **23**: 418-424 [PMID: 20862393 DOI: 10.1358/dnp.2010.23.7.1468395]
  - 367 **Novo Nordisk A/S**. Multiple Dose Trial Examining Dose Range, Escalation and Efficacy of Oral Semaglutide in Subjects With Type 2 Diabetes. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). [accessed 2016 May 26]. Available from: URL: <https://clinicaltrials.gov/ct2/show/NCT01923181> NLM Identifier: NCT01923181
  - 368 **Novo Nordisk A/S**. Efficacy and Long-term Safety of Oral Semaglutide Versus Sitagliptin in Subjects With Type 2 Diabetes (PIONEER 3). [accessed 2016 May 26]. Available from: URL: <https://clinicaltrials.gov/ct2/show/NCT02607865> NLM Identifier: NCT02607865
  - 369 **Khedkar A**, Iyer H, Anand A, Verma M, Krishnamurthy S, Savale S, Atigal A. A dose range finding study of novel oral insulin (IN-105) under fed conditions in type 2 diabetes mellitus subjects. *Diabetes Obes Metab* 2010; **12**: 659-664 [PMID: 20590742 DOI: 10.1111/j.1463-1326.2010.01213.x]
  - 370 **Eldor R**, Arbib E, Corcos A, Kidron M. Glucose-reducing effect of the ORMD-0801 oral insulin preparation in patients with uncontrolled type 1 diabetes: a pilot study. *PLoS One* 2013; **8**: e59524 [PMID: 23593142 DOI: 10.1371/journal.pone.0059524]
  - 371 **Zambrowicz B**, Freiman J, Brown PM, Frazier KS, Turnage A, Bronner J, Ruff D, Shadoan M, Banks P, Mseeh F, Rawlins DB, Goodwin NC, Mabon R, Harrison BA, Wilson A, Sands A, Powell DR. LX4211, a dual SGLT1/SGLT2 inhibitor, improved glycemic control in patients with type 2 diabetes in a randomized, placebo-controlled trial. *Clin Pharmacol Ther* 2012; **92**: 158-169 [PMID: 22739142 DOI: 10.1038/clpt.2012.58]
  - 372 **Powell DR**, Smith M, Greer J, Harris A, Zhao S, DaCosta C, Mseeh F, Shadoan MK, Sands A, Zambrowicz B, Ding ZM. LX4211 increases serum glucagon-like peptide 1 and peptide YY levels by reducing sodium/glucose cotransporter 1 (SGLT1)-mediated absorption of intestinal glucose. *J Pharmacol Exp Ther* 2013; **345**: 250-259 [PMID: 23487174 DOI: 10.1124/jpet.113.203364]
  - 373 **Powell DR**, DaCosta CM, Smith M, Doree D, Harris A, Buhning L, Heydorn W, Nouraldean A, Xiong W, Yalamanchili P, Mseeh F, Wilson A, Shadoan M, Zambrowicz B, Ding ZM. Effect of LX4211 on glucose homeostasis and body composition in preclinical models. *J Pharmacol Exp Ther* 2014; **350**: 232-242 [PMID: 24849925 DOI: 10.1124/jpet.114.214304]
  - 374 **Nuffer W**, Trujillo JM, Ellis SL. Technosphere insulin (Afrezza): a new, inhaled prandial insulin. *Ann Pharmacother* 2015; **49**: 99-106 [PMID: 25313261 DOI: 10.1177/1060028014554648]
  - 375 **Lupascu FG**, Dash M, Samal SK, Dubrue P, Lupusoru CE, Lupusoru RV, Dragostin O, Profire L. Development, optimization and biological evaluation of chitosan scaffold formulations of new xanthine derivatives for treatment of type-2 diabetes mellitus. *Eur J Pharm Sci* 2015; **77**: 122-134 [PMID: 26079402 DOI: 10.1016/j.ejps.2015.06.008]
  - 376 **Myers SA**. Zinc transporters and zinc signaling: new insights into their role in type 2 diabetes. *Int J Endocrinol* 2015; **2015**: 167503 [PMID: 25983752 DOI: 10.1155/2015/167503]
  - 377 **Pirags V**, Lebovitz H, Fouqueray P. Imeglimin, a novel glimin oral antidiabetic, exhibits a good efficacy and safety profile in type 2 diabetic patients. *Diabetes Obes Metab* 2012; **14**: 852-858 [PMID: 22519919 DOI: 10.1111/j.1463-1326.2012.01611.x]

- 378 **Vial G**, Chauvin MA, Bendridi N, Durand A, Meugnier E, Madec AM, Bernoud-Hubac N, Pais de Barros JP, Fontaine É, Acquaviva C, Hallakou-Bozec S, Bolze S, Vidal H, Rieusset J. Imeglimin normalizes glucose tolerance and insulin sensitivity and improves mitochondrial function in liver of a high-fat, high-sucrose diet mice model. *Diabetes* 2015; **64**: 2254-2264 [PMID: 25552598 DOI: 10.2337/db14-1220]
- 379 **Pacini G**, Mari A, Fouqueray P, Bolze S, Roden M. Imeglimin increases glucose-dependent insulin secretion and improves  $\beta$ -cell function in patients with type 2 diabetes. *Diabetes Obes Metab* 2015; **17**: 541-545 [PMID: 25694060 DOI: 10.1111/dom.12452]
- 380 **Fouqueray P**, Pirags V, Inzucchi SE, Bailey CJ, Scherthaner G, Diamant M, Lebovitz HE. The efficacy and safety of imeglimin as add-on therapy in patients with type 2 diabetes inadequately controlled with metformin monotherapy. *Diabetes Care* 2013; **36**: 565-568 [PMID: 23160726 DOI: 10.2337/dc12-0453]
- 381 **Fouqueray P**, Pirags V, Diamant M, Scherthaner G, Lebovitz HE, Inzucchi SE, Bailey CJ. The efficacy and safety of imeglimin as add-on therapy in patients with type 2 diabetes inadequately controlled with sitagliptin monotherapy. *Diabetes Care* 2014; **37**: 1924-1930 [PMID: 24722500 DOI: 10.2337/dc13-2349]
- 382 **Poxel SA**. A study of the efficacy and safety of 4 doses of imeglimin after 24 weeks of treatment in subjects with type 2 diabetes. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). [accessed 2016 Mar 22]. Available from: URL: <https://clinicaltrials.gov/ct2/show/NCT01951235> NLM Identifier: NCT01951235
- 383 **Gowda N**, Dandu A, Singh J, Biswas S, Raghav V, Lakshmi MN, Shilpa PC, Sunil V, Reddy A, Sadasivuni M, Aparna K, Verma MK, Moolemath Y, Anup MO, Venkataranganna MV, Somesh BP, Jagannath MR. Treatment with CNX-011-67, a novel GPR40 agonist, delays onset and progression of diabetes and improves beta cell preservation and function in male ZDF rats. *BMC Pharmacol Toxicol* 2013; **14**: 28 [PMID: 23692921 DOI: 10.1186/2050-6511-14-28]
- 384 **Sunil V**, Verma MK, Oommen AM, Sadasivuni M, Singh J, Vijayraghav DN, Chandravanshi B, Shetty J, Biswas S, Dandu A, Moolemath Y, Venkataranganna MV, Somesh BP, Jagannath MR. CNX-011-67, a novel GPR40 agonist, enhances glucose responsiveness, insulin secretion and islet insulin content in n-STZ rats and in islets from type 2 diabetic patients. *BMC Pharmacol Toxicol* 2014; **15**: 19 [PMID: 24666736 DOI: 10.1186/2050-6511-15-19]
- 385 **Burant CF**, Viswanathan P, Marcinak J, Cao C, Vakilynejad M, Xie B, Leifke E. TAK-875 versus placebo or glimepiride in type 2 diabetes mellitus: a phase 2, randomised, double-blind, placebo-controlled trial. *Lancet* 2012; **379**: 1403-1411 [PMID: 22374408 DOI: 10.1016/S0140-6736(11)61879-5]
- 386 **Morikawa T**, Akaki J, Ninomiya K, Kinouchi E, Tanabe G, Pongpiriyadacha Y, Yoshikawa M, Muraoka O. Salacinol and related analogs: new leads for type 2 diabetes therapeutic candidates from the Thai traditional natural medicine *Salacia chinensis*. *Nutrients* 2015; **7**: 1480-1493 [PMID: 25734563 DOI: 10.3390/nu7031480]

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## Evidence for current diagnostic criteria of diabetes mellitus

Ritesh Kumar, Lakshmana Perumal Nandhini, Sadishkumar Kamalanathan, Jayaprakash Sahoo, Muthupillai Vivekanadan

Ritesh Kumar, Lakshmana Perumal Nandhini, Sadishkumar Kamalanathan, Jayaprakash Sahoo, Muthupillai Vivekanadan, Department of Endocrinology and Metabolism, Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry 605006, India

**Author contributions:** Kumar R, Nandhini LP, Kamalanathan S, Sahoo J and Vivekanadan M contributed to conception and design, drafting the article and final approval of the manuscript.

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**Correspondence to:** Jayaprakash Sahoo, Assistant Professor, Department of Endocrinology and Metabolism, Jawaharlal Institute of Postgraduate Medical Education and Research, Dhanvantari Nagar, Pondicherry 605006, India. [jppgi@yahoo.com](mailto:jppgi@yahoo.com)  
 Telephone: +91-0413-2297374  
 Fax: +91-0413-2272067

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### Abstract

Diabetes mellitus is a non-communicable metabolic

derangement afflicting several millions of individuals globally. It is associated with several micro and macro-vascular complications and is also a leading cause of mortality. The unresolved issue is that of definition of the diagnostic threshold for diabetes. The World Health Organization and the American Diabetes Association (ADA) have laid down several diagnostic criteria for diagnosing diabetes and prediabetes based on the accumulating body of evidence. This review has attempted to analyse the scientific evidence supporting the justification of these differing criteria. The evidence for diagnosing diabetes is strong, and there is a concordance between the two professional bodies. The controversy arises when describing the normal lower limit of fasting plasma glucose (FPG) with little evidence favouring the reduction of the FPG by the ADA. Several studies have also shown the development of complications specific for diabetes in patients with prediabetes as defined by the current criteria though there is a significant overlap of such prevalence in individuals with normoglycemia. Large multinational longitudinal prospective studies involving subjects without diabetes and retinopathy at baseline will ideally help identify the threshold of glycemic measurements for future development of diabetes and its complications.

**Key words:** Diabetes; Prediabetes; Post glucose; Microvascular complications; Macrovascular complications

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**Core tip:** The diagnostic criteria for diabetes and prediabetes have evolved along the timeline taking into account new evidences which had developed. The major professional bodies have converged on to a consensus in developing the different thresholds for diagnosis of diabetes and associated states. Nevertheless, controversy remains on certain issues. There

is need to review the evolution of these criteria, the logistics behind their adoption and their association with different complications.

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## INTRODUCTION

Diabetes mellitus (DM) is a classic non-communicable disease that contributes to morbidity, mortality and poor quality of life apart from imposing economic burden on the health care system. The prevalence of type 2 DM is rising steadfast at an alarming rate and is estimated to affect 592 million individuals globally by the year 2035<sup>[1]</sup>. The International Diabetes Federation projections of the prevalence of prediabetes are expected to reach 471 million by 2035<sup>[1]</sup>. It is essential to make an early diagnosis and begin intervention to avoid complications of DM. But, defining the diagnostic threshold for diabetes and prediabetes has been a matter of intense debate. In this regard, several professional bodies have published differing diagnostic criteria over the last few decades. Below, is a review of the evolution of various diagnostic criteria and their validity.

## EVOLUTION OF DIAGNOSTIC CRITERIA FOR DIABETES

In ancient times, DM was diagnosed by tasting urine. Then the diagnosis was made by estimation of glucose in urine. But urine glycosuria did not correlate with glucose level in blood and was replaced by estimation of plasma glucose.

### **World Health Organization criteria (1965)**

The World Health Organization (WHO) in 1965 proposed the first widely accepted laboratory standard for diagnosing DM (Table 1). The committee recommended diagnosing DM in persons under the age of 45 years if 2 h venous plasma glucose was  $\geq 7.22$  mmol/L after loading with oral glucose of 50 or 100 g<sup>[2]</sup>. In persons aged more than 45 years, the committee considered that other clinical data should be the main guide to the diagnosis. Borderline state was defined if 2 h plasma glucose level was between 6.11 to 7.17 mmol/L.

### **National Diabetes Data Group criteria (1979)**

The National Diabetes Data Group (NDDG) in 1979 proposed new diagnostic criteria for DM<sup>[3]</sup>. It was based on the bimodal distribution of plasma glucose observed in Pima Indians and Nauruan population and the risk of

progression to DM and its complications<sup>[4,5]</sup>. A subject was diagnosed as having DM if fasting plasma glucose (FPG) was  $\geq 7.78$  mmol/L and/or 2-h plasma glucose (2-h PG) after 75 g of glucose was  $\geq 11.11$  mmol/L.

A study on Pima Indians revealed that the 2-h PG level differentiated those with DM from those without<sup>[4]</sup>. Subjects fall into two groups, one with a distribution of 2-h PG levels below 11.11 mmol/L, and the other with a distribution above 13.33 mmol/L. Diabetic retinopathy was mainly confined to the second group, i.e., in subjects whose 2-h PG level  $\geq 13.33$  mmol/L and this value divided the subjects with diabetes from nondiabetics. This bimodal distribution was further confirmed in Nauruan population<sup>[5]</sup>. Similar bimodal distribution also exists for FPG, where the glycemic threshold of about 7.78 mmol/L divides the two groups. Later, the bimodal glycemic distribution was reconfirmed from other populations with a high prevalence of DM like Mexican Americans<sup>[6]</sup>, Pacific Islanders<sup>[7]</sup>, South African Indians<sup>[8]</sup>, Egyptians<sup>[9]</sup>, Malaysians<sup>[10]</sup> and Americans in the United States<sup>[11]</sup>. However, for some populations, no such bimodality could be documented<sup>[12]</sup>.

With accumulating evidence from further studies, it was recognized that several individuals had 2-h PG levels that were intermediate between the normal and diabetic range. This group of individuals had 1%-5% risk of progression to DM per year though the majority continued to remain in this state and a few reverted to normalcy. It was also noted that there was an increased prevalence of atherosclerotic disease and electrocardiographic abnormalities and death in this population. This provided a window of opportunity to identify such individuals to intervene early and prevent progression to DM and its complications. To lay emphasis on this, the terminology "impaired glucose tolerance" (IGT) was first introduced by the NDDG of the National Institute of Health, United States. It was defined as a state of having venous FPG level of less than 7.8 mmol/L and a 2-h PG oral glucose tolerance test (OGTT) value between 7.8 mmol/L and 11.1 mmol/L<sup>[3]</sup>.

This group also aimed to standardize the protocol for OGTT internationally and recommended using 75 g of anhydrous glucose load for testing in nonpregnant adults. This was based on the observation that 50-g dose was not adequate in many individuals to identify IGT detected using the larger dose. Also, 100-g dose resulted in significant nausea in several study subjects. In subjects without diabetes it was reported that 50 g or 100 g result in approximately similar plasma glucose levels, the only difference was that 2 h PG was 0.83 mmol/L higher for 100 g as compared to 50 g oral glucose load<sup>[13,14]</sup>. Also there were no significant differences between 75- and 100-g doses. But in subjects with IGT there was higher difference (up to 2.78 mmol/L) in 2-h PG value between the 50 and 100 g oral glucose<sup>[3]</sup>.

### **WHO criteria (1980 and 1985)**

The WHO technical recommendation released in 1980



**Table 1** Evolution of diagnostic criteria of diabetes mellitus

	WHO 1965	WHO 1980	WHO 1985	ADA1997 WHO 1999	ADA 2003	IEC 2009 ADA 2010 WHO 2011
IFG	Not defined	Not defined	Not defined	Fasting ≥ 6.11 to < 7 mmol/L and post glucose (if measured) < 11.1 mmol/L	Fasting ≥ 5.5 to < 7 mmol/L and post glucose (if measured) < 11.1 mmol/L	Fasting ≥ 5.5 to < 7 mmol/L and post glucose (if measured) < 11.1 mmol/L or HbA1c (5.7%-6.4%)
IGT	Post glucose 6.11-7.1 mmol/L	Fasting < 8 mmol/L and/or post glucose ≥ 8 to < 11.1 mmol/L	Fasting < 7.8 mmol/L and/or post glucose ≥ 7.8 to < 11.1 mol/L	Fasting (if measured) < 7 mmol/L and post glucose ≥ 7.8 to 11.1 mmol/L	Fasting (if measured) < 7 mmol/L and post glucose 7.8 to 11.1 mmol/L	Fasting (if measured) < 7 mmol/L and post glucose 7.8 to 11.1 mmol/L or HbA1c (5.7%-6.4%)
DM	Post glucose ≥ 7.22 mmol/L	Fasting ≥ 8 mmol/L and/or post glucose ≥ 11.1 mmol/L	Fasting ≥ 7.8 mmol/L and/or post glucose ≥ 11.1 mmol/L	Fasting ≥ 7 mmol/L and/or post glucose ≥ 11.1 mmol/L	Fasting ≥ 7 mmol/L and/or post glucose ≥ 11.1 mmol/L	Fasting ≥ 7 mmol/L or Post glucose ≥ 11.1 mmol/L and/or HbA1c ≥ 6.5%

IFG: Impaired fasting glucose; IGT: Impaired glucose tolerance; DM: Diabetes mellitus; IEC: International Expert Committee; ADA: American Diabetes Association; WHO: World Health Organization; HbA1c: Glycated hemoglobin.

modified the criteria for diagnosing DM (Table 1). A venous FPG value above 8 mmol/L and a post glucose load 2-h PG value above 11 mmol/L were considered diagnostic of DM. This 2-h PG value was chosen based on observations that specific complications of DM rarely developed below this threshold. The term "IGT" suggested by the NDDG was also endorsed by WHO and became a part of the recommendation<sup>[15]</sup>. This was further slightly modified in the subsequent recommendations in 1985 and fasting and 2-h post glucose load venous plasma glucose thresholds were redefined as 7.8 mmol/L and 11.1 mmol/L respectively (Table 1)<sup>[16]</sup>.

#### **American Diabetes Association criteria (1997) and WHO criteria (1999)**

In 1997, the American Diabetes Association (ADA) lowered the threshold for FPG from 7.8 to 7.0 mmol/L and the 2-h post glucose load value was retained (Table 1)<sup>[17]</sup>. Impaired fasting glucose (IFG) was defined as FPG ≥ 6.1 mmol/L and < 7.0 mmol/L (Table 1). WHO adopted these criteria for the diagnosis of diabetes and prediabetes in 1999. In the second National Health and Nutrition Examination Survey (NHANES-II), only 26% of people with newly diagnosed DM by 1985 WHO had FPG ≥ 7.8 mmol/L, whereas 97% had 2-h PG ≥ 11.1 mmol/L<sup>[18]</sup>. Other studies also reported that as many as 80% of DM cases discovered in population screening by OGTT have FPG < 7.8 mmol/L<sup>[19-25]</sup>. Thus, the cutpoint of FPG > 7.8 mmol/L defined a greater degree of hyperglycemia than did the cutpoint of 2-h PG > 11.1 mmol/L. Thus, FPG appeared to be an insensitive test in population screening for undiagnosed DM.

This revision of the diagnostic criteria for the FPG from 7.8 to 7.0 mmol/L was based on the assumption that the threshold of the FPG and 2-h PG should identify

similar conditions. In Pima Indians<sup>[26]</sup>, Egyptians<sup>[9]</sup> and NHANES-III, both FPG and 2-h PG were strongly associated with retinopathy. The cutpoint for the 2-h PG was justified largely because of the dramatic increase in the prevalence of retinopathy approximately around that point. The equivalent cutpoint of FPG for 2-h PG level predicting retinopathy was computed in population studies of the Pima Indians, Egyptians, Pacific population, and NHANES III participants.

#### **ADA criteria (2003)**

A controversial change was brought out in the 2003 ADA guidelines, and it was the reduction in the cut-off point for defining the upper limit of FPG (Table 1). Based on four population-based epidemiological studies, the ideal cut-off point was shown to fall between 5.22-5.72 mmol/L and based on this data, an arbitrary cut-off of 5.55 mmol/L was chosen as the new threshold<sup>[27]</sup>. The lower threshold value of IFG was reduced from 6.11 to 5.55 mmol/L.

The phases of IFG and IGT represent metabolic states intermediate between normal glucose homeostasis and diabetic hyperglycaemia. The physiological basis of IFG and IGT are different. IFG is associated with insulin resistance at liver while IGT is associated with peripheral insulin resistance, at the level of skeletal muscle. The rationale for establishing the intermediate categories of impaired glucose regulation was based on their ability to predict future diabetes and its complications. The idea behind selecting the lower limit of IFG would be the identification of a threshold of FPG at which the risk of development of DM and complication or metabolic rises sharply. Data from Mauritius<sup>[28]</sup> and DECODE study<sup>[29]</sup> indicate that such a threshold of FPG does not exist for cardiovascular risk factors, all-cause mortality, or future DM. This criterion was based on

receiver operating characteristics (ROC) curve analyses of Pima Indian, Mauritius, San Antonio and Hoorn study data, which identified the baseline FPG levels, which maximised sensitivity and specificity for predicting DM over a 5-year period<sup>[25]</sup>. The ROC curve analyses indicated that a cut-point of 5.4-5.5 mmol/L gives the best combination of sensitivity and specificity for predicting future DM.

#### ADA criteria (2010) and WHO criteria (2011)

International Expert Committee (IEC 2009)<sup>[30]</sup>, ADA in 2010 and WHO in 2011 recommended a glycated hemoglobin (HbA1c) level of  $\geq 6.5\%$  as a diagnostic cut-off for DM (Table 1). HbA1c level reflects the average plasma glucose level over preceding three months. HbA1c is more convenient than glucose because it does not require fasting samples and is also not affected by recent changes in diet or activity. Another limitation of plasma glucose assay is lack of consistent accuracy of assay<sup>[31]</sup>. HbA1c has a greater analytic stability and less day-to-day variability in comparison to plasma glucose<sup>[32]</sup>. Selvin *et al*<sup>[33]</sup> evaluated the variabilities of glycemic measurement and found that 2-h PG levels [within-person coefficient of variation (CV), 16.7%; 95%CI: 15.0-18.3] and FPG (CV, 5.7%; 95%CI: 5.3-6.1) had substantially more variability compared with HbA1c (CV, 3.6%; 95%CI: 3.2-4.0) levels.

### HbA1c VS GLUCOSE CUTPOINTS FOR DIAGNOSIS OF DM

Lorenzo *et al*<sup>[34]</sup> compared 1999 WHO (2-h PG  $\geq 11.11$  mmol/L) and 2003 ADA criteria (FPG  $\geq 7$  mmol/L) with an HbA1c of  $\geq 6.5\%$ . It was found that sensitivity of HbA1c is poorer than plasma glucose because HbA1c diagnosed 5.2% of subjects as having diabetes compared to FPG (7.1%) and the 2-h PG (15.4%). Kramer *et al*<sup>[35]</sup> reported the sensitivity and specificity of HbA1c cutoff of 6.5% were 44% and 79% respectively based on the Rancho Bernardo Study. According to the ADA criteria, for this given HbA1c cut point of 6.5%, 85% of participants were classified as nondiabetic. Olson *et al*<sup>[36]</sup> compared HbA1c and standard OGTT for diagnosis of DM in three datasets from the prospective Screening for IGT study ( $n = 1581$ ), NHANES-III ( $n = 2014$ ), and NHANES 2005-2006 ( $n = 1111$ ) and reported that HbA1c criterion failed to recognize upto 70% of cases of DM. In conclusion, from above studies, HbA1c had the least sensitivity for diagnosis of DM in comparison to FPG and 2-h PG. Several studies have shown that HbA1c levels, as the plasma glucose levels, can predict the development of future DM<sup>[37,38]</sup>.

A limitation of HbA1c is that it is affected by red blood cell disorders<sup>[39]</sup>. Another limitation of HbA1c is that its levels depend on genetic factors<sup>[40,41]</sup>. It also suffers from analytic imprecision if methods other than high-performance liquid chromatography is used

for estimation and if such tests are not standardized. Measurement of HbA1c is currently well standardized with the adaptation of "national glycohemoglobin standardization program" protocols.

### OPTIMAL THRESHOLD OF THE HbA1c FOR RETINOPATHY

The most important question is how well HbA1c predicts retinopathy. IEC suggested a cutoff of the HbA1c of 6.5% for the diagnosis of DM because it was presumed that diabetic retinopathy sharply increased above this level. Unfortunately, most of the studies are cross-sectional and only a few prospective studies looked at the relationship between HbA1c and retinopathy (Table 2). Longitudinal prospective studies with subjects without DM and retinopathy at baseline will ideally give the association of HbA1c with retinopathy.

### CROSS-SECTIONAL STUDIES OF HbA1c FOR RETINOPATHY PREDICTION

Colagiuri *et al*<sup>[42]</sup> analysed the pooled data of nine studies and find that diabetes-specific retinopathy (after exclusion of mild retinopathy) was observed over the HbA1c range of 6.3% to 6.7% based on vignitile distribution and 6.4% by ROC analysis. He concluded that HbA1c  $\geq 6.5\%$  is a suitable alternative diagnostic criterion for DM. In the Australian Diabetes Obesity and Lifestyle study (AusDiab), retinopathy was assessed in 2182 participants aged  $\geq 25$  years. DM was not excluded in this study. The thresholds for increasing the prevalence of retinopathy was 6.1% for HbA1c<sup>[43]</sup>. Sabanayagam *et al*<sup>[44]</sup> examined the relationship of HbA1c to retinopathy in population-based sample of 3190 Malay adults aged 40-80 years in Singapore. HbA1c cut-off point of 6.6% detected mild retinopathy [87.0% sensitivity, 77.1% specificity and area under curve (AUC) 0.899] and 7.0% detected moderate retinopathy (82.9% sensitivity, 82.3% specificity and AUC 0.904). The prevalence of mild and moderate retinopathy was  $< 1\%$  below the optimal cut-off points. Xin *et al*<sup>[45]</sup> in Chinese population and Cho *et al*<sup>[46]</sup> in South Korean population found a threshold of 6.5% for detection of retinopathy. In ARIC study<sup>[47]</sup>, lower AUC was found (0.561 for any retinopathy, 0.543 for mild retinopathy and 0.658 for moderate) to severe retinopathy. These studies show that though there is an association between HbA1c and retinopathy, an optimal threshold could not be established.

### LONGITUDINAL STUDIES OF HbA1c FOR RETINOPATHY PREDICTION

Tsugawa *et al*<sup>[48]</sup> analyzed longitudinal data of 19897 Japanese adults who underwent a health checkup in 2006 and were followed up three years later. Logistic

**Table 2** Longitudinal studies assessing the glycated hemoglobin thresholds for retinopathy

Ref.	Study population characteristics	Assessment of retinopathy	Method of determining cutoff	Cut off
Tsugawa <i>et al</i> <sup>[48]</sup>	3 yr follow-up; number = 19987 Japanese subjects; age ≥ 21 yr; diabetes not excluded	Nonmydriatic 45° retinal photograph	Test for nonlinearity in multivariate logistic regression models with restricted cubic spline Multivariate logistic regression with categories of HbA1c as independent variable	Possible threshold at HbA1c levels between 6% and 7% 6.5%-6.9%
van Leiden <i>et al</i> <sup>[49]</sup> Hoorn study	7.9-11.0 yr follow-up; number = 233; age 50-74 yr; analyses in total study group and in subjects without diabetes	Ophthalmoscopy and fundus photography	Logistic model with categories of HbA1c (adjusted for age, sex, hypertension, glucose metabolism category)	No threshold found

HbA1c: Glycated hemoglobin.

regression analysis found that individuals with HbA1c levels of 6.5%-6.9% were at significantly higher risk of developing retinopathy at 3 years compared with those with HbA1c levels of 5.0%-5.4% [adjusted odds ratio, 2.35 (95%CI: 1.08-5.11)]. The incidence of retinopathy was determined in 233 individuals, aged 50 to 74 years, by ophthalmoscopy and fundus photography at baseline and after an average follow-up of 9.4 years in the Hoorn study<sup>[49]</sup>. Incidence of retinopathy was found to be significantly increased for HbA1c ranging between 5.8%-13.1% compared to HbA1c between 4.3%-5.2% but no optimal threshold of HbA1c was determined as the number of subjects in the study was not adequate.

Thresholds of HbA1c for retinopathy differ widely in the studies because of several reasons. First, different statistical methods were used in different studies. For example, in AusDiab study<sup>[43]</sup>, the cutoff was 6.1% by visual inspection, but cutoff was changed when change-point models were used. Without any adjustment, a threshold of 5.2% was calculated by using a change point model. After adjustment for age, sex and systolic blood pressure, the threshold for HbA1c was observed at 5.6% (95%CI: 3.9-6.2,  $P = 0.092$ ) and after further adjustment for diabetes duration, the threshold rose to 6.0% (3.9-7.0,  $P = 0.064$ ). Study on Pima Indians, Egyptians and in DETECT-2 study, cutoff of HbA1c were determined without any adjustment. Second, the threshold of HbA1c depends widely on the definition of retinopathy. Mild retinopathy is not specific for DM as it has been documented in non diabetic individuals too. Thresholds of HbA1c for mild, moderate and severe retinopathy can differ. For example, in Malay population thresholds of HbA1c were 6.6% and 7.0% respectively for mild and moderate retinopathy<sup>[44]</sup>. Also, the criteria for grading of retinopathy was different in different studies.

Third, the distributions of HbA1c may not be the same for different ethnicities. For example, Tsugawa *et al*<sup>[41]</sup> in cross-sectional study examined the relationships between a HbA1c level and the prevalence of retinopathy in black and white United States adults. Two thousand eight hundred and four white persons and 1008 black persons above 40 years of age were included in the study. After adjustment for age, sex,

hypertension, body mass index (BMI), family history of DM, and use of antidiabetes medications or insulin, the lowest HbA1c category for which the prevalence of retinopathy was significantly higher than the reference category (< 5.5%) was 6.0% to 6.4% for white persons (risk difference, 4.8% 95%CI: 0.5%-9.1%) and 5.5% to 5.9% for black persons (risk difference, 5.3%CI: 1.0%-9.5%). It was noted that the prevalence of retinopathy was higher at a lower HbA1c level in black Americans when compared white Americans. However, Bower *et al*<sup>[50]</sup> did not find any ethnic differences in the relationship of HbA1c with retinopathy in non-Hispanic white, non-Hispanic black and Hispanic American participants aged ≥ 40 years from the 2005-2008 NHANES. Finally, differences in threshold of HbA1c might be due to lack of standardization of HbA1c measurements, especially in older studies.

## HBA1C AND MACROVASCULAR COMPLICATIONS

Chronic hyperglycemia is a risk factor for adverse cardiovascular outcomes and mortality. A meta-analysis of 26 prospective studies assessed the association between HbA1c and major cardiovascular outcomes including all-cause mortality, incident cardiovascular diseases (CVD), CVD mortality, incident stroke and peripheral arterial disease. Only studies that followed up patients for more than 5 years were included. It was found that for every 1% increase in HbA1c, there was a 15% increase in hazard of all-cause mortality, 25% increase in CVD mortality, 17% in CVD, 17% in fatal coronary heart diseases and 29% increase in peripheral vascular diseases<sup>[51]</sup>. A positive dose response relationship was also noticed between HbA1c and the outcome measures and HbA1c was independent risk factor for adverse cardiovascular outcomes. Similar findings were noted in another meta-analysis by Selvin *et al*<sup>[52]</sup>.

## PREDIABETES AND RISK OF COMPLICATIONS

The association of complications is not restricted to

glucose levels above the diabetic threshold. It is a continuum, which continues in IGT and IFG range. Indeed, complications have also been documented in normal population, although of diminished magnitude. Various studies have looked into the paradigm of prediabetes forecasting the risks of micro and macro-vascular complications of diabetes.

## PREDIABETES AND RISK OF DIABETIC RETINOPATHY

The occurrence of microvascular complications associated with established DM is well known. However, such complications of dysglycemia have also been noted in patients who currently fall within the spectrum of prediabetes. The Diabetes Prevention Programme followed up individuals known to have prediabetes and analysed a subset of them for development of diabetic retinopathy. Eight percent of patients had evidence of retinopathy as defined as Early Treatment Diabetic Retinopathy Study (ETDRS) level 20<sup>[53]</sup>. One percent of the study population noted to have mild/moderate diabetic retinopathy as defined by ETDRS level 35-43. The Blue mountains eye study, a population-based survey of common eye diseases conducted in Australia, screened 3275 participants without DM for retinopathy lesions using six field fundus photographs. Microaneurysms were seen in 6.8% of nondiabetic population<sup>[54]</sup>. These studies defined retinopathy based on the presence or absence of microaneurysms, and it is to be noted that they are not specific for diabetic retinopathy and may occur in patients with systemic hypertension. In some studies, they have been shown to be related to atherosclerosis and carotid disease.

A population-based cross sectional survey of prevalence of DM, risk factors and associated conditions was done in the AusDiab study<sup>[55]</sup>. All participants detected to have DM and prediabetes and few with normal glucose tolerance (as defined by WHO 1999 criteria) were screened for retinopathy. Fundus photographs included two fields per eye, namely the macula and nasal to disc were graded according to Wisconsin criteria. The prevalence of diabetic retinopathy was 6.7% (95%CI: 5.3%-8.4%) in patients with prediabetes<sup>[56]</sup>. The prevalence of retinopathy was 5.8% in the population with normal glucose tolerance (95%CI: 3.7%-8.5%)<sup>[57]</sup>.

The Gutenberg health study, is a prospective population-based observational study conducted in a single centre in Germany that initially included 15010 individuals with the aim of studying ocular, cardiovascular, psychosomatic and immune disorders. A sub-cohort of 5000 individuals were analyzed to study the prevalence of retinopathy in those diagnosed to have prediabetes as defined by HbA1c value ranging from 5.7%-6.4% and its association with cardiovascular risk factors. Twenty two percent of participants were diagnosed to have prediabetes based on the HbA1c criteria. Eighty three percent of those with prediabetes were assessed for evidence of retinopathy by 3-field fundus photograph,

and 8.2% were found to have diabetic retinopathy. None of the participants had evidence of proliferative diabetic retinopathy. Though there was no statistically significant difference in the prevalence of cardiovascular risk factors between those with and without retinopathy, the number of participants with retinopathy was too small to draw any conclusion<sup>[58]</sup>.

## DIABETIC RETINOPATHY CHANGES IN NORMOGLYCEMIA

However, the retinal vascular changes seen in diabetic patients, termed isolated retinopathy signs, are often seen in individuals without DM or hypertension. The prevalence of these signs has been documented to range between 2.6%-8.6% in individuals without DM or hypertension. Such isolated retinopathy signs are often transient and on follow-up of these individuals, 40%-70% of such signs may resolve spontaneously<sup>[59,60]</sup>.

The Beaver Dam Eye study was a cross-sectional population-based study that investigated the association between retinopathy lesions and hypertension among non-diabetic individuals. Among the 4926 persons examined, 7.8% had evidence of retinopathy, and there was a significant association with systemic hypertension<sup>[61]</sup>. Similar prevalence was also seen in the Blue Mountains eye study where 3654 individuals from Sydney, Australia were screened for retinopathy using six field fundus photography. Retinal hemorrhages and microaneurysms were noted in 9.9% of individuals, and a significant positive relationship was noted between retinopathy and hypertension. However, DM was defined based on the FPG level > 7.8 mmol/L alone which could have resulted in mislabeling a significant proportion of individuals with DM as non-diabetics according to the current definitions<sup>[54]</sup>.

A follow-up of this cohort, where 2335 persons were re-examined reported a cumulative 5 year incidence of retinopathy as 9.7% and no significant association was found between incident retinopathy and blood glucose level or hypertension. The lack of a demonstrable association with hypertension could have resulted from inadequate power of the study. Among those with retinopathy at baseline, 3.5% had developed DM during the intervening five year period, and the retinopathy lesions had regressed or remained unchanged in 72.3%<sup>[59]</sup>. The ARIC study had reported the three-year incidence of retinopathy in non-diabetic subjects as 2.9% and also showed an association between retinopathy and hypertension and fasting blood glucose levels. Forty-three percent of any retinopathy signs seen among patients at baseline had regressed at the end of three years. This was found to be related to lower levels of cardiovascular risk factors<sup>[60]</sup>.

Whether these changes of retinopathy signify an increased risk of progression to DM is debatable. Most studies have shown no such association. However, retinopathy was predictive of incident DM in persons with a positive family history of DM during the follow-



up of the ARIC cohort. The incidence of DM was 10.4% among those with a family history of DM compared to 4.8% among those without a positive family history after a follow up of 3 years<sup>[62]</sup>. Similarly, the Beaver Dam study assessed the 15-year cumulative incidence of DM and hypertension among those with evidence of any retinopathy at baseline and found a significant association between incident DM and retinopathy among those < 65 years of age (24.3% vs 11.1%)<sup>[63]</sup>.

## PREDIABETES AND RISK OF NEPHROPATHY

The prevalence of nephropathy is increased in individuals diagnosed to have prediabetes compared to normal individuals. The NHANES data analysis revealed the prevalence of chronic kidney disease (CKD) (as defined by glomerular filtration rate (GFR) using "modification of diet in renal diseases" equation) in newly detected prediabetes to be 17.1% compared to 11.8% in those without DM and 24.2% in newly detected DM, after adjustment for age, gender and race. However, the diagnosis of prediabetes was based on measurement of FPG alone which could have underestimated the prevalence of prediabetes in the study. The other important risk factor for CKD, namely hypertension was documented based on self-reporting by study participants which could have again biased the results of the study. Nevertheless, the prevalence of CKD increased across the spectrum of dysglycemia<sup>[64]</sup>.

Few studies have shown that early kidney injury characterized by hyperfiltration is seen in those with prediabetes. Among the 1560 individuals included in the Renal Iohexol Clearance Survey in Tromsø 6 (RENIS-T6) study, it was seen that individuals with IFG had evidence of hyperfiltration (defined as GFR > 90<sup>th</sup> percentile determined by Iohexol method and adjusted for age, weight, height and use of renin-angiotensin inhibitors) when compared to those with normal glucose<sup>[65]</sup>. Similar results were obtained in the Huaian Diabetes Prevention program from China, where 5431 subjects were included to analyze the association between HbA1c level and renal hyperfiltration. The study had reported a positive correlation between HbA1c level and hyperfiltration independent of other parameters like age, sex, hypertension, BMI and lipid profile. The odds of hyperfiltration was 2.34 times higher in persons with HbA1c level of 6.21%-6.49% compared to those with A1c < 5.7%<sup>[66]</sup>. This indicates that chronic hyperglycemia is associated with hyperfiltration in addition to the acute effect of hyperglycemia that has been even in healthy subjects<sup>[67]</sup>.

Microalbuminuria, another marker of kidney injury, has also been found to be associated with prediabetic state. A study from New Zealand determined the prevalence of microalbuminuria and its association with other risk factors like ethnicity, glycemic status, hypertension, obesity and life style factors. Individuals with IGT had a higher prevalence of microalbuminuria

when compared to those with normal glycemic status (16.1% vs 4.0%) and glycemic status was found to be the most important determining factor of microalbuminuria in multivariate regression analysis<sup>[68]</sup>. However, contrary to the results of the above-mentioned studies, a study from Korea did not find any significant association between microalbuminuria and prediabetes. Forty-five percent of participants were diagnosed to have prediabetes based on ADA criteria for FPG and HbA1c in the Fifth Korea National Health and Nutrition Examination Survey (KNHANES V). Though the prevalence of microalbuminuria was higher in the prediabetic group when compared to the normal group (6.3% vs 3.6%), this difference was not seen following the adjustment for hypertension<sup>[69]</sup>.

## PREDIABETES AND RISK OF NEUROPATHY

Nerve conduction study conducted in 58 subjects from India with prediabetes as defined by the WHO criteria detected evidence of neuropathy in 32.8% of subjects which was evaluated by quantitative sensory testing (QST) and autonomic function tests. Autonomic neuropathy was evident in 8% of individuals, and QST was abnormal in 27.6% of subjects<sup>[70]</sup>.

## PREDIABETES AND RISK OF CARDIOVASCULAR DISEASES

Both IGT and IFG are associated with an increased risk of developing adverse cardiac events. A few studies have shown that patients with IGT have a greater risk when compared to patients with IFG. The risk also seems comparable to those with DM. Individuals with prediabetes were shown to have evidence of subclinical arteriosclerosis as measured by cardio-ankle vascular index (CAVI) in a recent study from Japan. CAVI is a sensitive indicator of arterial wall stiffness that is independent of blood pressure changes<sup>[71]</sup>. The odds of having high CAVI score among those with prediabetes was 1.29 (95%CI: 1.11-1.48) in men and 1.14 (95%CI: 1.01-1.28) for women compared to 2.41 (95%CI: 1.97-2.95) in men and 2.52 (95%CI: 1.94-3.28) for women with DM<sup>[72]</sup>.

Subclinical myocardial infarctions, defined as those unrecognized by the patient and the physicians are harbingers of major cardiovascular events in the future. The multi-ethnic study of atherosclerosis was instituted to study the prevalence and progression of subclinical cardiovascular disease in a population-based cohort from the United States<sup>[73]</sup>. In this cohort, the prevalence of unrecognized myocardial infarction detected based on electrocardiographic changes was found to be higher among those with IFG when compared to those with normal fasting glucose level (3.5% vs 1.4%) and this relationship persisted even after adjusting for other confounding risk factors<sup>[74]</sup>.

Increased risk of cardiovascular disease and all-cause mortality with abnormal glucose metabolism was documented in the AusDiab study after a median follow-up of 5.2 years. IFG was found to be an independent predictor of CVD mortality with a hazard ratio of 2.5 (95%CI: 1.2-5.1) after adjusting for other risk factors for CVD. However, IGT was not found to be associated with increased CVD mortality<sup>[75]</sup>.

A meta-analysis of studies evaluating the risk of coronary artery disease (CAD) associated with IFG as defined by the ADA and the WHO included 17 prospective studies. The risk of CAD was found to be increased in participants with IFG as defined by both criteria. The relative risk of CAD with IFG was 1.11 (95%CI: 1.02-1.21) using the ADA criteria and was 1.18 (95%CI: 1.10-1.28) when applying the WHO criteria. However, sub group analysis showed that the increased risk of CAD with IFG was not seen in studies that had excluded individuals with elevated 2-h plasma glucose. And further, the risk of CAD with IFG was not found to be significant when adjusted for other CAD risk factors<sup>[76]</sup>. A similar meta-analysis of studies analyzing the risk of stroke with prediabetes, an increased risk was seen in those studies which had defined prediabetes according to the WHO criteria (FPG 6.11-6.94 mmol/L). The risk was found to be increased in those with IGT and those with both IGT and IFG<sup>[77]</sup>.

## CONCLUSION

Current diagnostic criteria for DM or intermediate hyperglycemia is based on threshold of FPG, 2-h PG and HbA1c for diabetic complications, especially retinopathy. Controversies in diagnostic criteria are due to differences in inclusion criteria, different ethnic populations being studied, background prevalence of DM, definition of retinopathy used and statistical methods utilized. Therefore, there is a need to adopt uniform methodologies in studies across the globe to get universally comparable and interpretable results. Possibly, large longitudinal prospective studies involving subjects from different ethnicities, without diabetes and retinopathy at baseline will ideally help to identify the threshold of glycemic measurements (FPG, 2 h-PG and HbA1c) for future development of diabetes and its complications. Definition of retinopathy especially related to diabetes must be standardized universally. Further research is needed to understand better the pathophysiology of IFG and IGT. It is not well understood whether IFG and IGT are distinct metabolic abnormalities or they are parts of continuum. The factors predicting the development of future diabetes and its complications from IGT and IFG is also not well understood. This risk might be better assessed by the use of prediction scores which are weighted according to the glycemic measurements, other risk factors, and clinical features including complications. Finally, the extent to which future DM and its complications, especially cardiovascular diseases can be prevented

by adoption of modification of thresholds are not yet known.

New data from properly designed studies may help in revision of diagnostic criteria in future.

## REFERENCES

- 1 **Guariguata L**, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JE. Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes Res Clin Pract* 2014; **103**: 137-149 [PMID: 24630390 DOI: 10.1016/j.diabres.2013.11.002]
- 2 Diabetes mellitus. Report of a WHO expert committee. *World Health Organ Tech Rep Ser* 1965; **310**: 1-44 [PMID: 4953441]
- 3 Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. National Diabetes Data Group. *Diabetes* 1979; **28**: 1039-1057 [PMID: 510803 DOI: 10.2337/diab.28.12.1039]
- 4 **Bennett PH**, Rushforth NB, Miller M, LeCompte PM. Epidemiologic studies of diabetes in the Pima Indians. *Recent Prog Horm Res* 1976; **32**: 333-376 [PMID: 986678 DOI: 10.1016/b978-0-12-571132-6.50021-x]
- 5 **Zimmet P**, Whitehouse S. Bimodality of fasting and two-hour glucose tolerance distributions in a Micronesian population. *Diabetes* 1978; **27**: 793-800 [PMID: 680406 DOI: 10.2337/diab.27.8.793]
- 6 **Rosenthal M**, McMahan CA, Stern MP, Eifler CW, Haffner SM, Hazuda HP, Franco LJ. Evidence of bimodality of two hour plasma glucose concentrations in Mexican Americans: results from the San Antonio Heart study. *J Chronic Dis* 1985; **38**: 5-16 [PMID: 3972950 DOI: 10.1016/0021-9681(85)90003-7]
- 7 **Raper LR**, Taylor R, Zimmet P, Milne B, Balkau B. Bimodality in glucose tolerance distributions in the urban Polynesian population of Western Samoa. *Diabetes Res* 1984; **1**: 19-26 [PMID: 6529881]
- 8 **Omar MA**, Seedat MA, Dyer RB, Motala AA, Knight LT, Becker PJ. South African Indians show a high prevalence of NIDDM and bimodality in plasma glucose distribution patterns. *Diabetes Care* 1994; **17**: 70-73 [PMID: 8112193 DOI: 10.2337/diacare.17.1.70]
- 9 **Engelgau MM**, Thompson TJ, Herman WH, Boyle JP, Aubert RE, Kenny SJ, Badran A, Sous ES, Ali MA. Comparison of fasting and 2-hour glucose and HbA1c levels for diagnosing diabetes. Diagnostic criteria and performance revisited. *Diabetes Care* 1997; **20**: 785-791 [PMID: 9135943 DOI: 10.2337/diacare.20.5.785]
- 10 **Lim TO**, Bakri R, Morad Z, Hamid MA. Bimodality in blood glucose distribution: is it universal? *Diabetes Care* 2002; **25**: 2212-2217 [PMID: 12453963 DOI: 10.2337/diacare.25.12.2212]
- 11 **Fan J**, May SJ, Zhou Y, Barrett-Connor E. Bimodality of 2-h plasma glucose distributions in whites: the Rancho Bernardo study. *Diabetes Care* 2005; **28**: 1451-1456 [PMID: 15920067 DOI: 10.2337/diacare.28.6.1451]
- 12 **Hayner NS**, Kjelsberg MO, Epstein FH, Francis T. Carbohydrate tolerance and diabetes in a total community, tecumseh, michigan. 1. effects of age, sex, and test conditions on one-hour glucose tolerance in adults. *Diabetes* 1965; **14**: 413-423 [PMID: 14318589 DOI: 10.2337/diab.14.7.413]
- 13 **de Nobel E**, van't Laar A. The size of the loading dose as an important determinant of the results of the oral glucose tolerance test: a study in subjects with slightly impaired glucose tolerance. *Diabetes* 1978; **27**: 42-48 [PMID: 620880 DOI: 10.2337/diab.27.1.42]
- 14 **Sisk CW**, Burnham CE, Stewart J, McDonald GW. Comparison of the 50 and 100 gram oral glucose tolerance test. *Diabetes* 1970; **19**: 852-862 [PMID: 5480738 DOI: 10.2337/diab.19.11.852]
- 15 WHO Expert Committee on Diabetes Mellitus: second report. *World Health Organ Tech Rep Ser* 1980; **646**: 1-80 [PMID: 6771926]
- 16 Diabetes mellitus. Report of a WHO Study Group. *World Health Organ Tech Rep Ser* 1985; **727**: 1-113 [PMID: 3934850]
- 17 Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 1997; **20**: 1183-1197 [PMID: 9203460 DOI: 10.2337/diacare.20.7.1183]

- 18 **Harris MI**, Hadden WC, Knowler WC, Bennett PH. International criteria for the diagnosis of diabetes and impaired glucose tolerance. *Diabetes Care* 1985; **8**: 562-567 [PMID: 4075941 DOI: 10.2337/diacare.8.6.562]
- 19 **Modan M**, Halkin H, Karasik A, Lusky A. Effectiveness of glycosylated hemoglobin, fasting plasma glucose, and a single post load plasma glucose level in population screening for glucose intolerance. *Am J Epidemiol* 1984; **119**: 431-444 [PMID: 6702817]
- 20 **Modan M**, Harris MI. Fasting plasma glucose in screening for NIDDM in the U.S. and Israel. *Diabetes Care* 1994; **17**: 436-439 [PMID: 7741837 DOI: 10.2337/diacare.17.5.436]
- 21 **Finch CF**, Zimmet PZ, Alberti KG. Determining diabetes prevalence: a rational basis for the use of fasting plasma glucose concentrations? *Diabet Med* 1990; **7**: 603-610 [PMID: 2146068 DOI: 10.1111/j.1464-5491.1990.tb01457.x]
- 22 **Haffner SM**, Rosenthal M, Hazuda HP, Stern MP, Franco LJ. Evaluation of three potential screening tests for diabetes mellitus in a biethnic population. *Diabetes Care* 1984; **7**: 347-353 [PMID: 6468231 DOI: 10.2337/diacare.7.4.347]
- 23 **Blunt BA**, Barrett-Connor E, Wingard DL. Evaluation of fasting plasma glucose as screening test for NIDDM in older adults. Rancho Bernardo Study. *Diabetes Care* 1991; **14**: 989-993 [PMID: 1797513 DOI: 10.2337/diacare.14.11.989]
- 24 **Taylor R**, Zimmet P. Limitation of fasting plasma glucose for the diagnosis of diabetes mellitus. *Diabetes Care* 1981; **4**: 556-558 [PMID: 7347665 DOI: 10.2337/diacare.4.5.556]
- 25 **Hanson RL**, Nelson RG, McCance DR, Beart JA, Charles MA, Pettitt DJ, Knowler WC. Comparison of screening tests for non-insulin-dependent diabetes mellitus. *Arch Intern Med* 1993; **153**: 2133-2140 [PMID: 8379805 DOI: 10.1001/archinte.1993.00410180083010]
- 26 **McCance DR**, Hanson RL, Charles MA, Jacobsson LT, Pettitt DJ, Bennett PH, Knowler WC. Comparison of tests for glycated haemoglobin and fasting and two hour plasma glucose concentrations as diagnostic methods for diabetes. *BMJ* 1994; **308**: 1323-1328 [PMID: 8019217 DOI: 10.1136/bmj.308.6940.1323]
- 27 **Genuth S**, Alberti KG, Bennett P, Buse J, DeFronzo R, Kahn R, Kitzmiller J, Knowler WC, Lebovitz H, Lernmark A, Nathan D, Palmer J, Rizza R, Saudek C, Shaw J, Steffes M, Stern M, Tuomilehto J, Zimmet P. Follow-up report on the diagnosis of diabetes mellitus. *Diabetes Care* 2003; **26**: 3160-3167 [PMID: 14578255 DOI: 10.2337/diacare.26.11.3160]
- 28 **Shaw JE**, Zimmet PZ, Hodge AM, de Courten M, Dowse GK, Chitson P, Tuomilehto J, Alberti KG. Impaired fasting glucose: how low should it go? *Diabetes Care* 2000; **23**: 34-39 [PMID: 10857965 DOI: 10.2337/diacare.23.1.34]
- 29 **Forouhi NG**, Balkau B, Borch-Johnsen K, Dekker J, Glumer C, Qiao Q, Spijkerman A, Stolk R, Tabac A, Wareham NJ. The threshold for diagnosing impaired fasting glucose: a position statement by the European Diabetes Epidemiology Group. *Diabetologia* 2006; **49**: 822-827 [PMID: 16525842 DOI: 10.1007/s00125-006-0189-4]
- 30 **Gillett MJ**. International Expert Committee report on the role of the A1c assay in the diagnosis of diabetes: *Diabetes Care* 2009; **32**(7): 1327-1334. *Clin Biochem Rev* 2009; **30**: 197-200 [PMID: 20011212]
- 31 **Gambino R**. Glucose: a simple molecule that is not simple to quantify. *Clin Chem* 2007; **53**: 2040-2041 [PMID: 18267929 DOI: 10.1373/clinchem.2007.094466]
- 32 **Petersen PH**, Jørgensen LG, Brandslund I, De Fine Olivarius N, Stahl M. Consequences of bias and imprecision in measurements of glucose and HbA1c for the diagnosis and prognosis of diabetes mellitus. *Scand J Clin Lab Invest Suppl* 2005; **240**: 51-60 [PMID: 16112960 DOI: 10.1080/00365510500236135]
- 33 **Selvin E**, Crainiceanu CM, Brancati FL, Coresh J. Short-term variability in measures of glycemia and implications for the classification of diabetes. *Arch Intern Med* 2007; **167**: 1545-1551 [PMID: 17646610 DOI: 10.1001/archinte.167.14.1545]
- 34 **Lorenzo C**, Haffner SM. Performance characteristics of the new definition of diabetes: the insulin resistance atherosclerosis study. *Diabetes Care* 2010; **33**: 335-337 [PMID: 19880585 DOI: 10.2337/dc09-1357]
- 35 **Kramer CK**, Araneta MR, Barrett-Connor E. A1C and diabetes diagnosis: The Rancho Bernardo Study. *Diabetes Care* 2010; **33**: 101-103 [PMID: 19837792 DOI: 10.2337/dc09-1366]
- 36 **Olson DE**, Rhee MK, Herrick K, Ziemer DC, Twombly JG, Phillips LS. Screening for diabetes and pre-diabetes with proposed A1C-based diagnostic criteria. *Diabetes Care* 2010; **33**: 2184-2189 [PMID: 20639452 DOI: 10.2337/dc10-0433]
- 37 **Nakagami T**, Tajima N, Oizumi T, Karasawa S, Wada K, Kameda W, Susa S, Kato T, Daimon M. Hemoglobin A1c in predicting progression to diabetes. *Diabetes Res Clin Pract* 2010; **87**: 126-131 [PMID: 19945760 DOI: 10.1016/j.diabres.2009.11.001]
- 38 **Bae JC**, Rhee EJ, Lee WY, Park SE, Park CY, Oh KW, Park SW, Kim SW. Optimal range of HbA1c for the prediction of future diabetes: a 4-year longitudinal study. *Diabetes Res Clin Pract* 2011; **93**: 255-259 [PMID: 21676480 DOI: 10.1016/j.diabres.2011.05.028]
- 39 **Dankner R**, Bergman M, Danoff A, Qureshi S, Whitford I, Kaviani N, Dynkevich Y, Roth J. The metabolic deterioration that antedates diabetes: personal trajectories of HbA(1c) and fasting glucose as early indicators and possible triggers for intervention. *Diabetes Metab Res Rev* 2013; **29**: 1-7 [PMID: 23175191 DOI: 10.1002/dmrr.2373]
- 40 **Mostafa SA**, Khunti K, Srinivasan BT, Webb D, Gray LJ, Davies MJ. The potential impact and optimal cut-points of using glycated haemoglobin, HbA1c, to detect people with impaired glucose regulation in a UK multi-ethnic cohort. *Diabetes Res Clin Pract* 2010; **90**: 100-108 [PMID: 20633944 DOI: 10.1016/j.diabres.2010.06.008]
- 41 **Tsugawa Y**, Mukamal KJ, Davis RB, Taylor WC, Wee CC. Should the hemoglobin A1c diagnostic cutoff differ between blacks and whites? A cross-sectional study. *Ann Intern Med* 2012; **157**: 153-159 [PMID: 22868832 DOI: 10.7326/0003-4819-157-3-201208070-00004]
- 42 **Colagiuri S**, Lee CM, Wong TY, Balkau B, Shaw JE, Borch-Johnsen K. Glycemic thresholds for diabetes-specific retinopathy: implications for diagnostic criteria for diabetes. *Diabetes Care* 2011; **34**: 145-150 [PMID: 20978099 DOI: 10.2337/dc10-1206]
- 43 **Tapp RJ**, Zimmet PZ, Harper CA, de Courten MP, McCarty DJ, Balkau B, Taylor HR, Welborn TA, Shaw JE. Diagnostic thresholds for diabetes: the association of retinopathy and albuminuria with glycaemia. *Diabetes Res Clin Pract* 2006; **73**: 315-321 [PMID: 16644057 DOI: 10.1016/j.diabres.2006.02.008]
- 44 **Sabanayagam C**, Liew G, Tai ES, Shankar A, Lim SC, Subramaniam T, Wong TY. Relationship between glycated haemoglobin and microvascular complications: is there a natural cut-off point for the diagnosis of diabetes? *Diabetologia* 2009; **52**: 1279-1289 [PMID: 19387611 DOI: 10.1007/s00125-009-1360-5]
- 45 **Xin Z**, Yuan MX, Li HX, Hua L, Feng JP, Shi J, Zhu XR, Cao X, Yang JK. Evaluation for fasting and 2-hour glucose and HbA1c for diagnosing diabetes based on prevalence of retinopathy in a Chinese population. *PLoS One* 2012; **7**: e40610 [PMID: 22808204 DOI: 10.1371/journal.pone.0040610]
- 46 **Cho NH**, Kim TH, Woo SJ, Park KH, Lim S, Cho YM, Park KS, Jang HC, Choi SH. Optimal HbA1c cutoff for detecting diabetic retinopathy. *Acta Diabetol* 2013; **50**: 837-842 [PMID: 23354926 DOI: 10.1007/s00592-013-0452-3]
- 47 **Selvin E**, Ning Y, Steffes MW, Bash LD, Klein R, Wong TY, Astor BC, Sharrett AR, Brancati FL, Coresh J. Glycated hemoglobin and the risk of kidney disease and retinopathy in adults with and without diabetes. *Diabetes* 2011; **60**: 298-305 [PMID: 20978092 DOI: 10.2337/db10-1198]
- 48 **Tsugawa Y**, Takahashi O, Meigs JB, Davis RB, Imamura F, Fukui T, Taylor WC, Wee CC. New diabetes diagnostic threshold of hemoglobin A(1c) and the 3-year incidence of retinopathy. *Diabetes* 2012; **61**: 3280-3284 [PMID: 22891221 DOI: 10.2337/db12-0103]
- 49 **van Leiden HA**, Dekker JM, Moll AC, Nijpels G, Heine RJ, Bouter LM, Stehouwer CD, Polak BC. Risk factors for incident retinopathy in a diabetic and nondiabetic population: the Hoorn study. *Arch Ophthalmol* 2003; **121**: 245-251 [PMID: 12583792]
- 50 **Bower JK**, Brancati FL, Selvin E. No ethnic differences in the association of glycated hemoglobin with retinopathy: the national



- health and nutrition examination survey 2005-2008. *Diabetes Care* 2013; **36**: 569-573 [PMID: 23069841 DOI: 10.2337/dc12-0404]
- 51 **Zhang Y**, Hu G, Yuan Z, Chen L. Glycosylated hemoglobin in relationship to cardiovascular outcomes and death in patients with type 2 diabetes: a systematic review and meta-analysis. *PLoS One* 2012; **7**: e42551 [PMID: 22912709 DOI: 10.1371/journal.pone.0042551]
  - 52 **Selvin E**, Marinopoulos S, Berkenblit G, Rami T, Brancati FL, Powe NR, Golden SH. Meta-analysis: glycosylated hemoglobin and cardiovascular disease in diabetes mellitus. *Ann Intern Med* 2004; **141**: 421-431 [PMID: 15381515 DOI: 10.7326/0003-4819-141-6-200409210-00007]
  - 53 **Diabetes Prevention Program Research Group**. The prevalence of retinopathy in impaired glucose tolerance and recent-onset diabetes in the Diabetes Prevention Program. *Diabet Med* 2007; **24**: 137-144 [PMID: 17257275 DOI: 10.1111/j.1464-5491.2007.02043.x]
  - 54 **Yu T**, Mitchell P, Berry G, Li W, Wang JJ. Retinopathy in older persons without diabetes and its relationship to hypertension. *Arch Ophthalmol* 1998; **116**: 83-89 [PMID: 9445212]
  - 55 **Dunstan DW**, Zimmet PZ, Welborn TA, Cameron AJ, Shaw J, de Courten M, Jolley D, McCarty DJ. The Australian Diabetes, Obesity and Lifestyle Study (AusDiab)--methods and response rates. *Diabetes Res Clin Pract* 2002; **57**: 119-129 [PMID: 12062857 DOI: 10.1016/S0168-8227(02)00025-6]
  - 56 **Wong TY**, Barr EL, Tapp RJ, Harper CA, Taylor HR, Zimmet PZ, Shaw JE. Retinopathy in persons with impaired glucose metabolism: the Australian Diabetes Obesity and Lifestyle (AusDiab) study. *Am J Ophthalmol* 2005; **140**: 1157-1159 [PMID: 16376677 DOI: 10.1016/j.ajo.2005.07.030]
  - 57 **Tapp RJ**, Shaw JE, Harper CA, de Courten MP, Balkau B, McCarty DJ, Taylor HR, Welborn TA, Zimmet PZ. The prevalence of and factors associated with diabetic retinopathy in the Australian population. *Diabetes Care* 2003; **26**: 1731-1737 [PMID: 12766102 DOI: 10.2337/diacare.26.6.1731]
  - 58 **Lamparter J**, Raum P, Pfeiffer N, Peto T, Höhn R, Elflein H, Wild P, Schulz A, Schneider A, Mirshahi A. Prevalence and associations of diabetic retinopathy in a large cohort of prediabetic subjects: the Gutenberg Health Study. *J Diabetes Complications* 1989; **28**: 482-487 [PMID: 24630763 DOI: 10.1016/j.jdiacomp.2014.02.008]
  - 59 **Cugati S**, Cikamatana L, Wang JJ, Kifley A, Liew G, Mitchell P. Five-year incidence and progression of vascular retinopathy in persons without diabetes: the Blue Mountains Eye Study. *Eye (Lond)* 2006; **20**: 1239-1245 [PMID: 16167076 DOI: 10.1038/sj.eye.6702085]
  - 60 **Wong TY**, Klein R, Amirul Islam FM, Cotch MF, Couper DJ, Klein BE, Hubbard LD, Sharrett AR. Three-year incidence and cumulative prevalence of retinopathy: the atherosclerosis risk in communities study. *Am J Ophthalmol* 2007; **143**: 970-976 [PMID: 17399675 DOI: 10.1016/j.ajo.2007.02.020]
  - 61 **Klein R**, Klein BE, Moss SE, Wang Q. Hypertension and retinopathy, arteriolar narrowing, and arteriovenous nicking in a population. *Arch Ophthalmol* 1994; **112**: 92-98 [PMID: 8285901]
  - 62 **Cugati S**, Mitchell P, Wang JJ. Do retinopathy signs in non-diabetic individuals predict the subsequent risk of diabetes? *Br J Ophthalmol* 2006; **90**: 928-929 [PMID: 16782966 DOI: 10.1136/bjo.2006.095943]
  - 63 **Klein R**, Klein BE, Moss SE, Wong TY. The relationship of retinopathy in persons without diabetes to the 15-year incidence of diabetes and hypertension: Beaver Dam Eye Study. *Trans Am Ophthalmol Soc* 2006; **104**: 98-107 [PMID: 17471330]
  - 64 **Plantinga LC**, Crews DC, Coresh J, Miller ER, Saran R, Yee J, Hedgeman E, Pavkov M, Eberhardt MS, Williams DE, Powe NR. Prevalence of chronic kidney disease in US adults with undiagnosed diabetes or prediabetes. *Clin J Am Soc Nephrol* 2010; **5**: 673-682 [PMID: 20338960 DOI: 10.2215/CJN.07891109]
  - 65 **Melsum T**, Mathisen UD, Ingebrechtsen OC, Jenssen TG, Njølstad I, Solbu MD, Toft I, Eriksen BO. Impaired fasting glucose is associated with renal hyperfiltration in the general population. *Diabetes Care* 2011; **34**: 1546-1551 [PMID: 21593291 DOI: 10.2337/dc11-0235]
  - 66 **Hu W**, Hao H, Yu W, Wu X, Zhou H. Association of elevated glycosylated hemoglobin A1c with hyperfiltration in a middle-aged and elderly Chinese population with prediabetes or newly diagnosed diabetes: a cross-sectional study. *BMC Endocr Disord* 2015; **15**: 47 [PMID: 26363801 DOI: 10.1186/s12902-015-0043-0]
  - 67 **Greene SA**, Dalton RN, Turner C, Haycock GB, Chantler C. Hyperglycemia with and without glycosuria: effect on inulin and para-amino hippurate clearance. *Kidney Int* 1987; **32**: 896-899 [PMID: 3430968 DOI: 10.1038/ki.1987.292]
  - 68 **Metcalfe PA**, Baker JR, Scragg RK, Dryson E, Scott AJ, Wild CJ. Microalbuminuria in a middle-aged workforce. Effect of hyperglycemia and ethnicity. *Diabetes Care* 1993; **16**: 1485-1493 [PMID: 8299438 DOI: 10.2337/diacare.16.11.1485]
  - 69 **Kim CH**, Kim KJ, Kim BY, Jung CH, Mok JO, Kang SK, Kim HK. Prediabetes is not independently associated with microalbuminuria in Korean general population: the Korea National Health and Nutrition Examination Survey 2011-2012 (KNHANES V-2,3). *Diabetes Res Clin Pract* 2014; **106**: e18-e21 [PMID: 25271114 DOI: 10.1016/j.diabres.2014.09.004]
  - 70 **Kannan MA**, Sarva S, Kandadai RM, Paturi VR, Jabeen SA, Borgohain R. Prevalence of neuropathy in patients with impaired glucose tolerance using various electrophysiological tests. *Neurol India* 2014; **62**: 656-661 [PMID: 25591680 DOI: 10.4103/0028-3886.149393]
  - 71 **Sun CK**. Cardio-ankle vascular index (CAVI) as an indicator of arterial stiffness. *Integr Blood Press Control* 2013; **6**: 27-38 [PMID: 23667317 DOI: 10.2147/IBPC.S34423]
  - 72 **Namekata T**, Shirai K, Tanabe N, Miyamishi K, Nakata M, Suzuki K, Arai C, Ishizuka N. Estimating the extent of subclinical arteriosclerosis of persons with prediabetes and diabetes mellitus among Japanese urban workers and their families: a cross-sectional study. *BMC Cardiovasc Disord* 2016; **16**: 52 [PMID: 26911293 DOI: 10.1186/s12872-016-0230-6]
  - 73 **Bild DE**, Bluemke DA, Burke GL, Detrano R, Diez Roux AV, Folsom AR, Greenland P, Jacob DR, Kronmal R, Liu K, Nelson JC, O'Leary D, Saad MF, Shea S, Szklo M, Tracy RP. Multi-Ethnic Study of Atherosclerosis: objectives and design. *Am J Epidemiol* 2002; **156**: 871-881 [PMID: 12397006 DOI: 10.1093/aje/kwf113]
  - 74 **Stacey RB**, Leaverton PE, Schocken DD, Peregoy JA, Bertoni AG. Prediabetes and the association with unrecognized myocardial infarction in the multi-ethnic study of atherosclerosis. *Am Heart J* 2015; **170**: 923-928 [PMID: 26542500 DOI: 10.1016/j.ahj.2015.08.003]
  - 75 **Barr EL**, Zimmet PZ, Welborn TA, Jolley D, Magliano DJ, Dunstan DW, Cameron AJ, Dwyer T, Taylor HR, Tonkin AM, Wong TY, McNeil J, Shaw JE. Risk of cardiovascular and all-cause mortality in individuals with diabetes mellitus, impaired fasting glucose, and impaired glucose tolerance: the Australian Diabetes, Obesity, and Lifestyle Study (AusDiab). *Circulation* 2007; **116**: 151-157 [PMID: 17576864 DOI: 10.1161/CIRCULATIONAHA.106.685628]
  - 76 **Xu T**, Liu W, Cai X, Ding J, Tang H, Huang Y, Hu Y. Risk of Coronary Heart Disease in Different Criterion of Impaired Fasting Glucose: A Meta-Analysis. *Medicine (Baltimore)* 2015; **94**: e1740 [PMID: 26448033 DOI: 10.1097/MD.0000000000001740]
  - 77 **Lee M**, Saver JL, Hong KS, Song S, Chang KH, Ovbiagele B. Effect of pre-diabetes on future risk of stroke: meta-analysis. *BMJ* 2012; **344**: e3564 [PMID: 22677795]

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## ***In vivo* corneal confocal microscopy in diabetes: Where we are and where we can get**

Ernesto Maddaloni, Francesco Sabatino

Ernesto Maddaloni, Unit of Endocrinology and Diabetes, Department of Medicine, University Campus Bio-Medico, 00128 Rome, Italy

Francesco Sabatino, Department of Ophthalmology, University Campus Bio-Medico, 00128 Rome, Italy

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**Correspondence to:** Ernesto Maddaloni, MD, Unit of Endocrinology and Diabetes, Department of Medicine, University Campus Bio-Medico, Via Alvaro del Portillo 21, 00128 Rome, Italy. [e.maddaloni@unicampus.it](mailto:e.maddaloni@unicampus.it)  
Telephone: +39-06-22541697  
Fax: +39-06-22541698

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### **Abstract**

*In vivo* corneal confocal microscopy (IVCCM) is a novel,

reproducible, easy and noninvasive technique that allows the study of the different layers of the cornea at a cellular level. As cornea is the most innervated organ of human body, several studies investigated the use of corneal confocal microscopy to detect diabetic neuropathies, which are invalidating and deadly complications of diabetes mellitus. Corneal nerve innervation has been shown impaired in subjects with diabetes and a close association between damages of peripheral nerves due to the diabetes and alterations in corneal sub-basal nerve plexus detected by IVCCM has been widely demonstrated. Interestingly, these alterations seem to precede the clinical onset of diabetic neuropathies, paving the path for prevention studies. However, some concerns still prevent the full implementation of this technique in clinical practice. In this review we summarize the most recent and relevant evidences about the use of IVCCM for the diagnosis of peripheral sensorimotor polyneuropathy and of autonomic neuropathy in diabetes. New perspectives and current limitations are also discussed.

**Key words:** Corneal confocal microscopy; Neuropathy; diabetes; Cornea; New technologies

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**Core tip:** Diabetic neuropathies are common, invalidating and often undiagnosed complications affecting a huge number of subjects with diabetes. *In vivo* corneal confocal microscopy is a novel, reproducible, easy and noninvasive technique that has been widely studied as a useful tool for the diagnosis of neuropathy. Promising data suggest its implementation in clinical and research practice will help to face the current health emergency related to nerve damages in diabetes.

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## INTRODUCTION

Diabetic neuropathies are common and invalidating, but often undiagnosed, complications affecting up to 50% of subjects with diabetes<sup>[1,2]</sup>. Diabetic neuropathies encompass a wide spectrum of clinical and pathophysiological frameworks characterized by a progressive loss of nerve fibers, which may affect both somatic and autonomic nerves. While the rates of myocardial infarction, stroke, low-extremity amputations and end-stage renal disease due to diabetes are declining<sup>[3]</sup>, this is not the case for trends in the incidence of neuropathies. An early diagnosis and correct staging of neuropathy is essential for risk stratification, therapeutic decisions and research purposes. To date screening and diagnosis of diabetic neuropathies mainly relies on symptoms questionnaires, clinical examination, quantitative sensory tests and reflex tests<sup>[4,5]</sup>. Nerve conduction studies should also be used to confirm the diagnosis and to assess the severity of the disease<sup>[2]</sup>. *In vivo* corneal confocal microscopy (IVCCM) is a non-invasive technique to visualize and analyze corneal anatomy at high magnification allowing the study of the different layers and cells of cornea, the most innervated organ of human body<sup>[6]</sup>. Several studies in the last decade investigated the use of IVCCM for the diagnosis of sensorimotor and, more recently, autonomic neuropathies<sup>[7]</sup>. In this review we aim to summarize the most recent and relevant evidences about IVCCM use in people with diabetes, focusing on strength and limitations future studies should overcome.

## CORNEAL INNERVATION

The eye has historically been considered as a pivotal organ for the study of diabetes-related complications. The transparency of the ocular structures has always been used as a diagnostic tool to investigate and actually see *in vivo* vascular changes in the retina. More recently, increasing attention has been paid to corneal nerve anatomy for the study of human neuropathies. The cornea is provided of the densest innervation within the body receiving nerve fibers from 50-450 sensory trigeminal neurons *via* the ophthalmic branch of trigeminal nerve<sup>[8,9]</sup>. These fibers travel above the choroid, reach the limbus where they organize into a nerve plexus<sup>[10]</sup>. Corneal stromal nerves derive from the limbal plexus and branch into fibers with smaller diameter that establish close connections with keratocytes and corneal epithelial cells<sup>[11]</sup>. The fibers become denser and smaller in diameter as they reach the corneal apex creating a sub-epithelial dense network in the epithelium, known as sub-basal corneal nerve plexus.



**Figure 1** Acquisition of the images with *in vivo* corneal confocal microscopy. Patient is comfortably seated in front of the machine whilst the operator advances the scanning probe against the cornea with a joystick.

## IVCCM

The introduction of IVCCM in ophthalmology (Figure 1) has represented a breakthrough for the study of ocular as well as systemic diseases since this diagnostic tool allows for a non-invasive and *in vivo* visualization of all the corneal layers, including nerves. There are currently three models of confocal microscope available, namely the slit-scanning, the tandem scanning and the laser scanning confocal microscope. They provide images with different resolution, contrast and magnification with high inter-device variability. However all of them result in high-resolution scans of the cornea. These features make IVCCM an ideal tool to investigate changes in corneal and ocular surface. It is used as an aid in the diagnosis and to monitor efficacy of therapies in different ocular diseases like, dry eyes, *Acanthamoeba* keratitis or keratoconus or following corneal surgery<sup>[12]</sup>. Several studies have also investigated the role of IVCCM as a diagnostic tool to be implemented for the assessment of several systemic diseases. Indeed, changes in the corneal sub-basal nerves have been shown to correlate with several neurodegenerative diseases, small fiber neuropathies, Fabry disease and other conditions causing peripheral neuropathy like diabetes, HIV-infection, genetic diseases, toxic drugs or autoimmune diseases<sup>[13]</sup>.

## IVCCM AND DIABETIC PERIPHERAL SENSORIMOTOR NEUROPATHY

Changes in corneal morphology including reduced thickness, thinner epithelium, irregular endothelium and reduction in corneal nerve bundles have been described in diabetes<sup>[14]</sup>. Therefore, qualitative and quantitative analysis of the sub-basal corneal nerve plexus by IVCCM

has been hypothesized to be a good method for the evaluation and quantification of nerve damages in people affected by diabetes. Several studies have been conducted to test this hypothesis in both type 1 and type 2 diabetes<sup>[7,15]</sup>. Overall, corneal nerve innervation has been shown impaired in subjects with diabetes, independently by the presence of overt neuropathy<sup>[16]</sup>. Interestingly, IVCCM was able in identifying early neuropathy also in subjects with pre-diabetes. Asghar *et al.*<sup>[17]</sup> assessed corneal innervation in thirty-seven subjects with impaired glucose tolerance showing that IVCCM, but not electrophysiology studies, detected signs of nerve damages which correlated with neuropathy symptoms, neurological deficits and intraepidermal nerve fiber density (IENFD).

A close association between damages of peripheral nerves due to the diabetes and alterations in corneal sub-basal nerve plexus detected with IVCCM has been widely demonstrated by a number of studies. A landmark small cross-sectional study by Malik *et al.*<sup>[18]</sup> showed corneal nerve fiber density, length and branch density were reduced in eighteen diabetic subjects with different grade neuropathy vs healthy controls, with a gradual reduction of these parameters with increasing neuropathy severity. Similarly, corneal nerve tortuosity was found increased in diabetics and in those with more severe neuropathy<sup>[19]</sup>. Subsequently several studies by this and other groups confirmed the efficacy of IVCCM for the identification of diabetic neuropathy in larger populations of type 1<sup>[20-23]</sup>, type 2 diabetes<sup>[24]</sup> or both<sup>[25-27]</sup>. In particular, baseline features of the population enrolled in the LANDMark study, the largest study testing IVCCM in 242 type 1 diabetes vs 154 controls, confirmed the reduced corneal nerve fiber length in those with neuropathy<sup>[22]</sup>. Longitudinal results of this study are not yet available.

As cornea is innervated by small A $\delta$  and C fibers, IVCCM was tested with good results as a surrogate marker of small fiber neuropathy<sup>[28]</sup>. Before IVCCM, the evaluation of IENFD by skin biopsy was the gold standard method to quantitatively assess small fiber damages, with obvious limitations for a routine implementation in clinical practice due to its invasiveness. A comparable diagnostic efficiency between IVCCM and IENFD in type 1 diabetes has been recently shown in a study by Chen *et al.*<sup>[29]</sup> where the area under the receiver operator curve for the identification of neuropathy did not significantly differ between the two techniques.

Among all the parameters of corneal innervation evaluated by IVCCM, the great majority of published studies agree about the validity and the overall good reproducibility of corneal nerve fiber density and length. In particular, the latter was shown to be the best predictor of diabetic sensorimotor polyneuropathy in 81 subjects affected by type 1 diabetes, with an optimized threshold for sensitivity and specificity at 14.0 mm/mm<sup>2</sup><sup>[30]</sup>. This threshold, however, has to be applied taking into account the natural age-dependent variation in corneal nerve fiber length. On the contrary, corneal

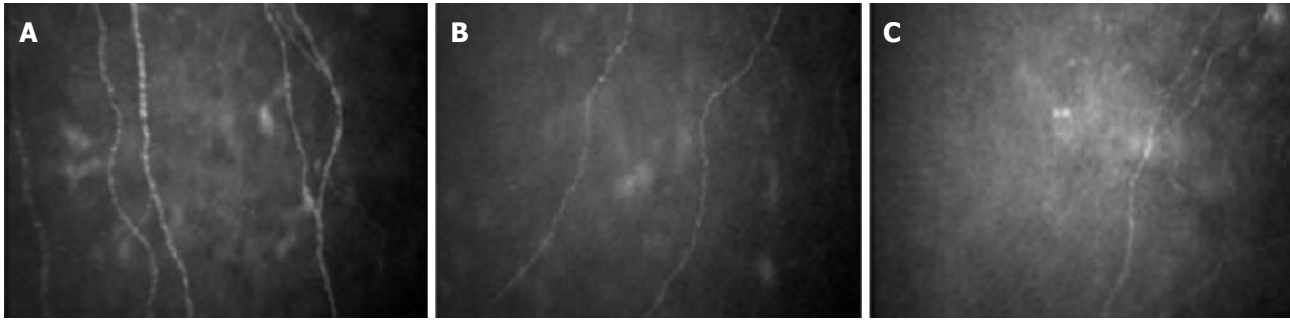
nerve beadings and tortuosity showed the highest inter and intra-individual variability, questioning the validity of these two measurements for the diagnosis of diabetic peripheral neuropathy<sup>[21]</sup>. However, the measurement of nerve tortuosity could be relevant to ameliorate the predictive value of fiber length, as tortuosity-standardized corneal nerve fiber length was better than non-standardized length in differentiating between individuals with and without neuropathy<sup>[31]</sup>.

## IVCCM AND DIABETIC AUTONOMIC NEUROPATHY

Diabetic autonomic neuropathy is a form of diabetic neuropathy that results from the damage of small (A $\delta$ , B and C) nerve fibers. It represents one of the most overlooked but life-threatening complications of diabetes, associated with gastrointestinal, genitourinary, vasomotor and cardiac symptoms. In particular cardiac autonomic neuropathy affects up to 40% of diabetic patients and is associated with silent myocardial ischemia, stroke and increased mortality<sup>[5]</sup>. Because of the structural similarity between the corneal nerve fibers analyzed with IVCCM and the small fibers conducting autonomic signals, IVCCM has been recently tested as a diagnostic tool for autonomic neuropathies. We showed that subjects affected by type 1 diabetes with cardiac autonomic neuropathy, as evaluated by cardiovascular autonomic reflex tests (CARTs), had reduced corneal nerve fiber density and length when compared to peers without cardiac autonomic neuropathy and to healthy controls, independently of the presence of peripheral neuropathy<sup>[32]</sup> (Figure 2). Subsequently, Tavakoli *et al.*<sup>[33]</sup> confirmed our observation in a population of both type 1 and type 2 diabetic subjects. Subjects were evaluated by the Composite Autonomic Symptom Scale (COMPASS), by CARTs, by sympathetic skin response, and by IVCCM. The Composite Autonomic Severity Score (CASS) was also calculated. Corneal nerve fiber density, length and branch density were significantly reduced in subjects with autonomic deficits than in those without. IVCCM showed moderate-to-strong correlations with COMPASS and CASS, with a good sensitivity and specificity for fiber length and fiber density for the diagnosis of diabetic autonomic neuropathy<sup>[33]</sup>. Similarly, corneal nerve innervation was found to be related to sudomotor function in subjects affected by type 2 diabetes<sup>[34]</sup>. Moreover, a significant correlation between corneal sensitivity and measures of cardiac autonomic function in subjects with type 1 diabetes was recently reported<sup>[35]</sup>. However, in the same study no significant relationship with sub-basal nerve density was found.

## IVCCM IN DIABETES: WHERE WE CAN GET

A growing literature supports IVCCM as an innovative



**Figure 2** Corneal innervation evaluated by *in vivo* corneal confocal microscopy in a health subjects (A), in a subject affected by type 1 diabetes without cardiac autonomic neuropathy (B) and in a subject affected by type 1 diabetes with cardiac autonomic neuropathy (C). Nerve fiber density and length is reduced in people with type 1 diabetes and in those with cardiac autonomic neuropathy.

technique helpful to face diabetic neuropathies, which are prevalent complications of diabetes and cause of high healthcare expenditures, reduced quality of life, high morbidity and mortality. In particular IVCCM could have possible implications for the prevention of diabetic neuropathies and for research studies about its pathophysiology and treatments.

### Prevention

Changes in the corneal sub-basal nerve plexus anticipate other clinical and electrophysiology signs of neuropathy<sup>[35,36]</sup>. Two longitudinal studies showed that lower corneal nerve fiber length predicts the onset of diabetic sensorimotor polyneuropathy in type 1 diabetic subjects followed-up for 3.5 and 4 years<sup>[37,38]</sup>. For the identification of new cases of neuropathy, the sensitivity and specificity of corneal nerve fiber length were 82% and 69%, respectively, with an optimal threshold of 14.9 mm/mm<sup>2</sup> in one study<sup>[37]</sup>, and 63% and 74% with an optimal threshold of 14.1 mm/mm<sup>2</sup> in the other one<sup>[38]</sup>. This suggests IVCCM allows the identification of at-risk patients to implement preventive strategies such as tight glycemic control and multifactorial interventions. Indeed, a prospective cohort study in subjects with type 1 diabetes without overt neuropathy showed that modifications in the corneal sub-basal nerve plexus over 4 years of follow-up were related to clinical and metabolic factors such as age, HbA1c and HDL cholesterol. This highlights the capability of IVCCM for monitoring the efficacy of preventive strategies aimed to modify the natural history of diabetic neuropathy before symptoms and signs become measurable by classical screening tests<sup>[39]</sup>.

Moreover, Petropoulos *et al.*<sup>[40]</sup> showed that degeneration of corneal nerve fibers are detectable before other microvascular complications appear, questioning whether early detection of small fibers distress by IVCCM could also work as a precocious surrogate marker for vascular risk stratification as well as micro-albuminuria or retinopathy. However, to date no studies have specifically investigated the predictive value of IVCCM with regards to the development of diabetic complications other than neuropathy.

### Research tool

Besides the clinical implications, IVCCM could be a useful research tool to investigate neuropathy pathophysiology. The promising data showing early degeneration of corneal nerve fibers in impaired glucose tolerance and new onset type 2 diabetes<sup>[17,24,41]</sup> support the hypothesis the pathophysiology of diabetic neuropathy starts very early in diabetes<sup>[42]</sup>. Overall the data discussed so far pose IVCCM as a research tool to investigate the first steps of neuropathy, where other methods such as nerve conduction studies are not enough sensitive to detect pathological changes. Moreover, even though corneal nerve fibers have sensitive but not autonomic function, the above reported results about the association of IVCCM and diabetic autonomic neuropathy overall suggest that molecular cascades eventually leading to the damage of the sub-basal corneal plexus could also occur to nerves with similar structure such as the autonomic ones, even if they have different function. They also suggest that IVCCM is a surrogate easy and non-invasive marker of autonomic dysfunction, which mostly remains undiagnosed because of scarce implementation of the recommended diagnostic tests. However, these promising findings have still to be tested for their usefulness for cardiovascular risk-stratification in larger and homogenous populations.

Interestingly, some reports show that IVCCM is also able in detecting regeneration of small nerve fiber after therapeutics. IVCCM showed significant improvements in nerve morphology after pancreas transplantation<sup>[43]</sup>, after simultaneous kidney-pancreas transplantation<sup>[44]</sup>, after improvements in risk factors for diabetic neuropathy<sup>[45]</sup>, in subjects treated with continuous subcutaneous insulin infusion<sup>[46]</sup> and in phase 2 studies<sup>[47]</sup>. These studies suggest IVCCM is novel noninvasive tool to establish early nerve repair consequent to medical intervention that is missed by current assessment techniques.

### Current pitfalls and limitations

Some pitfalls still exist about the use of IVCCM for the diagnosis of diabetic neuropathies. In particular, an age-related decline in corneal nerve fibers density



and length occurs, claiming for age-standardized normative values. Moreover, there are uncertainties about racial differences in corneal nerve measures. IVCCM implementation and utility is also limited by the time and the expertise required for image analysis. Some arguments against IVCCM also claim a scarce reproducibility of corneal nerve measurements. In this regard, it has been recently shown preservation in the inter- and intra-observer reproducibility of fiber length measurements when using a fully automated analysis program, which also eliminates the need for trained analyst personnel and reduces the analysis time<sup>[25,48]</sup>.

## CONCLUSION

In conclusion, IVCCM currently represents a fascinating link between laboratory and clinical sciences through which diabetic neuropathies can be analyzed by assessing nerve density, tortuosity and length. We acknowledge our conclusions may be limited by the fact this manuscript is not a systematic review. However, to limit a possible selection bias, we carefully search throughout the literature for human studies testing IVCCM in people with diabetes and reported both positive and negative results, strength and limitations. As a result of our search, we reported that several evidences support the role of IVCCM as an easy and non-invasive clinical and research tool for the study of diabetic neuropathies, but some limitations including bias in image selection, reproducibility and the required expertise to perform the scan and read the images still need to be fully addressed.

## REFERENCES

- 1 **Young MJ**, Boulton AJ, MacLeod AF, Williams DR, Sonksen PH. A multicentre study of the prevalence of diabetic peripheral neuropathy in the United Kingdom hospital clinic population. *Diabetologia* 1993; **36**: 150-154 [PMID: 8458529 DOI: 10.1007/BF00400697]
- 2 **Tesfaye S**, Boulton AJ, Dyck PJ, Freeman R, Horowitz M, Kempner P, Lauria G, Malik RA, Spallone V, Vinik A, Bernardi L, Valensi P. Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. *Diabetes Care* 2010; **33**: 2285-2293 [PMID: 20876709 DOI: 10.2337/dc10-1303]
- 3 **Gregg EW**, Li Y, Wang J, Burrows NR, Ali MK, Rolka D, Williams DE, Geiss L. Changes in diabetes-related complications in the United States, 1990-2010. *N Engl J Med* 2014; **370**: 1514-1523 [PMID: 24738668 DOI: 10.1056/NEJMoa1310799]
- 4 **Boulton AJ**, Malik RA, Arezzo JC, Soslenko JM. Diabetic somatic neuropathies. *Diabetes Care* 2004; **27**: 1458-1486 [PMID: 15161806 DOI: 10.2337/diacare.27.6.1458]
- 5 **Spallone V**, Ziegler D, Freeman R, Bernardi L, Frontoni S, Pop-Busui R, Stevens M, Kempner P, Hilsted J, Tesfaye S, Low P, Valensi P. Cardiovascular autonomic neuropathy in diabetes: clinical impact, assessment, diagnosis, and management. *Diabetes Metab Res Rev* 2011; **27**: 639-653 [PMID: 21695768 DOI: 10.1002/dmrr.1239]
- 6 **Jalbert I**, Stapleton F, Papas E, Sweeney DF, Coroneo M. In vivo confocal microscopy of the human cornea. *Br J Ophthalmol* 2003; **87**: 225-236 [PMID: 12543757 DOI: 10.1136/bjo.87.2.225]
- 7 **Papanas N**, Ziegler D. Corneal confocal microscopy: Recent progress in the evaluation of diabetic neuropathy. *J Diabetes Investig* 2015; **6**: 381-389 [PMID: 26221515 DOI: 10.1111/jdi.12335]
- 8 **Müller LJ**, Marfurt CF, Kruse F, Tervo TM. Corneal nerves: structure, contents and function. *Exp Eye Res* 2003; **76**: 521-542 [PMID: 12697417 DOI: 10.1016/S0014-4835(03)00050-2]
- 9 **Shaheen BS**, Bakir M, Jain S. Corneal nerves in health and disease. *Surv Ophthalmol* 2014; **59**: 263-285 [PMID: 24461367 DOI: 10.1016/j.survophthal.2013.09.002]
- 10 **Al-Aqaba MA**, Fares U, Suleman H, Lowe J, Dua HS. Architecture and distribution of human corneal nerves. *Br J Ophthalmol* 2010; **94**: 784-789 [PMID: 19889832 DOI: 10.1136/bjo.2009.173799]
- 11 **Müller LJ**, Pels L, Vrensen GF. Ultrastructural organization of human corneal nerves. *Invest Ophthalmol Vis Sci* 1996; **37**: 476-488 [PMID: 8595948]
- 12 **Patel DV**, McGhee CN. In vivo confocal microscopy of human corneal nerves in health, in ocular and systemic disease, and following corneal surgery: a review. *Br J Ophthalmol* 2009; **93**: 853-860 [PMID: 19019923 DOI: 10.1136/bjo.2008.150615]
- 13 **Wang EF**, Misra SL, Patel DV. In Vivo Confocal Microscopy of the Human Cornea in the Assessment of Peripheral Neuropathy and Systemic Diseases. *Biomed Res Int* 2015; **2015**: 951081 [PMID: 26770980 DOI: 10.1155/2015/951081]
- 14 **Rosenberg ME**, Tervo TM, Immonen IJ, Müller LJ, Grönholm-Riska C, Vesaluoma MH. Corneal structure and sensitivity in type 1 diabetes mellitus. *Invest Ophthalmol Vis Sci* 2000; **41**: 2915-2921 [PMID: 10967045]
- 15 **Papanas N**, Ziegler D. Corneal confocal microscopy: a new technique for early detection of diabetic neuropathy. *Curr Diab Rep* 2013; **13**: 488-499 [PMID: 23666893 DOI: 10.1007/s11892-013-0390-z]
- 16 **Mocan MC**, Durukan I, Ircek M, Orhan M. Morphologic alterations of both the stromal and subbasal nerves in the corneas of patients with diabetes. *Cornea* 2006; **25**: 769-773 [PMID: 17068451 DOI: 10.1097/01.icc.0000224640.58848.54]
- 17 **Asghar O**, Petropoulos IN, Alam U, Jones W, Jeziorska M, Marshall A, Ponirakis G, Fadavi H, Boulton AJ, Tavakoli M, Malik RA. Corneal confocal microscopy detects neuropathy in subjects with impaired glucose tolerance. *Diabetes Care* 2014; **37**: 2643-2646 [PMID: 24969581 DOI: 10.2337/dc14-0279]
- 18 **Malik RA**, Kallinikos P, Abbott CA, van Schie CH, Morgan P, Efron N, Boulton AJ. Corneal confocal microscopy: a non-invasive surrogate of nerve fibre damage and repair in diabetic patients. *Diabetologia* 2003; **46**: 683-688 [PMID: 12739016 DOI: 10.1007/s00125-003-1086-8]
- 19 **Kallinikos P**, Berhanu M, O'Donnell C, Boulton AJ, Efron N, Malik RA. Corneal nerve tortuosity in diabetic patients with neuropathy. *Invest Ophthalmol Vis Sci* 2004; **45**: 418-422 [PMID: 14744880 DOI: 10.1167/iovs.03-0637]
- 20 **Dehghani C**, Pritchard N, Edwards K, Vagenas D, Russell AW, Malik RA, Efron N. Natural history of corneal nerve morphology in mild neuropathy associated with type 1 diabetes: development of a potential measure of diabetic peripheral neuropathy. *Invest Ophthalmol Vis Sci* 2014; **55**: 7982-7990 [PMID: 25406279 DOI: 10.1167/iovs.14-15605]
- 21 **Sivaskandarajah GA**, Halpern EM, Lovblom LE, Weisman A, Orlov S, Bril V, Perkins BA. Structure-function relationship between corneal nerves and conventional small-fiber tests in type 1 diabetes. *Diabetes Care* 2013; **36**: 2748-2755 [PMID: 23579181 DOI: 10.2337/dc12-2075]
- 22 **Pritchard N**, Edwards K, Dehghani C, Fadavi H, Jeziorska M, Marshall A, Petropoulos IN, Ponirakis G, Russell AW, Sampson GP, Shahidi AM, Srinivasan S, Tavakoli M, Vagenas D, Malik RA, Efron N. Longitudinal assessment of neuropathy in type 1 diabetes using novel ophthalmic markers (LANDMark): study design and baseline characteristics. *Diabetes Res Clin Pract* 2014; **104**: 248-256 [PMID: 24629408 DOI: 10.1016/j.diabres.2014.02.011]
- 23 **Ishibashi F**, Okino M, Ishibashi M, Kawasaki A, Endo N, Kosaka A, Uetake H. Corneal nerve fiber pathology in Japanese type 1 diabetic patients and its correlation with antecedent glycemic control and blood pressure. *J Diabetes Investig* 2012; **3**: 191-198 [PMID: 24843565 DOI: 10.1111/j.2040-1124.2011.00157.x]
- 24 **Ziegler D**, Papanas N, Zhivov A, Allgeier S, Winter K, Ziegler I, Brüggemann J, Strom A, Peschel S, Köhler B, Stachs O, Guthoff

- RF, Roden M. Early detection of nerve fiber loss by corneal confocal microscopy and skin biopsy in recently diagnosed type 2 diabetes. *Diabetes* 2014; **63**: 2454-2463 [PMID: 24574045 DOI: 10.2337/db13-1819]
- 25 **Petropoulos IN**, Alam U, Fadavi H, Marshall A, Asghar O, Dabbah MA, Chen X, Graham J, Ponirakis G, Boulton AJ, Tavakoli M, Malik RA. Rapid automated diagnosis of diabetic peripheral neuropathy with in vivo corneal confocal microscopy. *Invest Ophthalmol Vis Sci* 2014; **55**: 2071-2078 [PMID: 24569580 DOI: 10.1167/iops.13-13787]
- 26 **Petropoulos IN**, Alam U, Fadavi H, Asghar O, Green P, Ponirakis G, Marshall A, Boulton AJ, Tavakoli M, Malik RA. Corneal nerve loss detected with corneal confocal microscopy is symmetrical and related to the severity of diabetic polyneuropathy. *Diabetes Care* 2013; **36**: 3646-3651 [PMID: 23877983 DOI: 10.2337/dc13-0193]
- 27 **Stem MS**, Hussain M, Lentz SI, Raval N, Gardner TW, Pop-Busui R, Shtein RM. Differential reduction in corneal nerve fiber length in patients with type 1 or type 2 diabetes mellitus. *J Diabetes Complications* 2014; **28**: 658-661 [PMID: 25044236 DOI: 10.1016/j.jdiacomp.2014.06.007]
- 28 **Quattrini C**, Tavakoli M, Jeziorska M, Kallinikos P, Tesfaye S, Finnigan J, Marshall A, Boulton AJ, Efron N, Malik RA. Surrogate markers of small fiber damage in human diabetic neuropathy. *Diabetes* 2007; **56**: 2148-2154 [PMID: 17513704 DOI: 10.2337/db07-0285]
- 29 **Chen X**, Graham J, Dabbah MA, Petropoulos IN, Ponirakis G, Asghar O, Alam U, Marshall A, Fadavi H, Ferdousi M, Azmi S, Tavakoli M, Efron N, Jeziorska M, Malik RA. Small nerve fiber quantification in the diagnosis of diabetic sensorimotor polyneuropathy: comparing corneal confocal microscopy with intraepidermal nerve fiber density. *Diabetes Care* 2015; **38**: 1138-1144 [PMID: 25795415 DOI: 10.2337/dc14-2422]
- 30 **Ahmed A**, Bril V, Orszag A, Paulson J, Yeung E, Ngo M, Orlov S, Perkins BA. Detection of diabetic sensorimotor polyneuropathy by corneal confocal microscopy in type 1 diabetes: a concurrent validity study. *Diabetes Care* 2012; **35**: 821-828 [PMID: 22323412 DOI: 10.2337/dc11-1396]
- 31 **Edwards K**, Pritchard N, Vagenas D, Russell A, Malik RA, Efron N. Standardizing corneal nerve fibre length for nerve tortuosity increases its association with measures of diabetic neuropathy. *Diabet Med* 2014; **31**: 1205-1209 [PMID: 24750318 DOI: 10.1111/dme.12466]
- 32 **Maddaloni E**, Sabatino F, Del Toro R, Crugliano S, Grande S, Lauria Pantano A, Maurizi AR, Palermo A, Bonini S, Pozzilli P, Manfrini S. In vivo corneal confocal microscopy as a novel non-invasive tool to investigate cardiac autonomic neuropathy in Type 1 diabetes. *Diabet Med* 2015; **32**: 262-266 [PMID: 25251450 DOI: 10.1111/dme.12583]
- 33 **Tavakoli M**, Begum P, McLaughlin J, Malik RA. Corneal confocal microscopy for the diagnosis of diabetic autonomic neuropathy. *Muscle Nerve* 2015; **52**: 363-370 [PMID: 25556884 DOI: 10.1002/mus.24553]
- 34 **Ishibashi F**, Kojima R, Kawasaki A, Yamanaka E, Kosaka A, Uetake H. Correlation between sudomotor function, sweat gland duct size and corneal nerve fiber pathology in patients with type 2 diabetes mellitus. *J Diabetes Investig* 2014; **5**: 588-596 [PMID: 25411628 DOI: 10.1111/jdi.12171]
- 35 **Misra SL**, Craig JP, Patel DV, McGhee CN, Pradhan M, Ellyett K, Kilfoyle D, Braatvedt GD. In Vivo Confocal Microscopy of Corneal Nerves: An Ocular Biomarker for Peripheral and Cardiac Autonomic Neuropathy in Type 1 Diabetes Mellitus. *Invest Ophthalmol Vis Sci* 2015; **56**: 5060-5065 [PMID: 26241393 DOI: 10.1167/iops.15-16711]
- 36 **Hossain P**, Sachdev A, Malik RA. Early detection of diabetic peripheral neuropathy with corneal confocal microscopy. *Lancet* 2005; **366**: 1340-1343 [PMID: 16226599 DOI: 10.1016/S0140-6736(05)67546-0]
- 37 **Lovblom LE**, Halpern EM, Wu T, Kelly D, Ahmed A, Boulet G, Orszag A, Ng E, Ngo M, Bril V, Perkins BA. In vivo corneal confocal microscopy and prediction of future incident neuropathy in type 1 diabetes: a preliminary longitudinal analysis. *Can J Diabetes* 2015; **39**: 390-397 [PMID: 25936902 DOI: 10.1016/j.cjcd.2015.02.006]
- 38 **Pritchard N**, Edwards K, Russell AW, Perkins BA, Malik RA, Efron N. Corneal confocal microscopy predicts 4-year incident peripheral neuropathy in type 1 diabetes. *Diabetes Care* 2015; **38**: 671-675 [PMID: 25573881 DOI: 10.2337/dc14-2114]
- 39 **Dehghani C**, Pritchard N, Edwards K, Russell AW, Malik RA, Efron N. Risk Factors Associated With Corneal Nerve Alteration in Type 1 Diabetes in the Absence of Neuropathy: A Longitudinal In Vivo Corneal Confocal Microscopy Study. *Cornea* 2016; **35**: 847-852 [PMID: 26845318 DOI: 10.1097/ICO.0000000000000760]
- 40 **Petropoulos IN**, Green P, Chan AW, Alam U, Fadavi H, Marshall A, Asghar O, Efron N, Tavakoli M, Malik RA. Corneal confocal microscopy detects neuropathy in patients with type 1 diabetes without retinopathy or microalbuminuria. *PLoS One* 2015; **10**: e0123517 [PMID: 25853247 DOI: 10.1371/journal.pone.0123517]
- 41 **Azmi S**, Ferdousi M, Petropoulos IN, Ponirakis G, Alam U, Fadavi H, Asghar O, Marshall A, Atkinson AJ, Jones W, Boulton AJ, Tavakoli M, Jeziorska M, Malik RA. Corneal Confocal Microscopy Identifies Small-Fiber Neuropathy in Subjects With Impaired Glucose Tolerance Who Develop Type 2 Diabetes. *Diabetes Care* 2015; **38**: 1502-1508 [PMID: 25877814 DOI: 10.2337/dc14-2733]
- 42 **Papanas N**, Vinik AI, Ziegler D. Neuropathy in prediabetes: does the clock start ticking early? *Nat Rev Endocrinol* 2011; **7**: 682-690 [PMID: 21750507 DOI: 10.1038/nrendo.2011.113]
- 43 **Mehra S**, Tavakoli M, Kallinikos PA, Efron N, Boulton AJ, Augustine T, Malik RA. Corneal confocal microscopy detects early nerve regeneration after pancreas transplantation in patients with type 1 diabetes. *Diabetes Care* 2007; **30**: 2608-2612 [PMID: 17623821 DOI: 10.2337/dc07-0870]
- 44 **Tavakoli M**, Mitu-Pretorian M, Petropoulos IN, Fadavi H, Asghar O, Alam U, Ponirakis G, Jeziorska M, Marshall A, Efron N, Boulton AJ, Augustine T, Malik RA. Corneal confocal microscopy detects early nerve regeneration in diabetic neuropathy after simultaneous pancreas and kidney transplantation. *Diabetes* 2013; **62**: 254-260 [PMID: 23002037 DOI: 10.2337/db12-0574]
- 45 **Tavakoli M**, Kallinikos P, Iqbal A, Herbert A, Fadavi H, Efron N, Boulton AJ, A Malik R. Corneal confocal microscopy detects improvement in corneal nerve morphology with an improvement in risk factors for diabetic neuropathy. *Diabet Med* 2011; **28**: 1261-1267 [PMID: 21699561 DOI: 10.1111/j.1464-5491.2011.03372.x]
- 46 **Azmi S**, Ferdousi M, Petropoulos IN, Ponirakis G, Fadavi H, Tavakoli M, Alam U, Jones W, Marshall A, Jeziorska M, Boulton AJ, Efron N, Malik RA. Corneal confocal microscopy shows an improvement in small-fiber neuropathy in subjects with type 1 diabetes on continuous subcutaneous insulin infusion compared with multiple daily injection. *Diabetes Care* 2015; **38**: e3-e4 [PMID: 25538321 DOI: 10.2337/dc14-1698]
- 47 **Brines M**, Dunne AN, van Velzen M, Proto PL, Ostenson CG, Kirk RI, Petropoulos IN, Javed S, Malik RA, Cerami A, Dahan A. ARA 290, a nonerythropoietic peptide engineered from erythropoietin, improves metabolic control and neuropathic symptoms in patients with type 2 diabetes. *Mol Med* 2014; **20**: 658-666 [PMID: 25387363 DOI: 10.2119/molmed.2014.00215]
- 48 **Ostrovski I**, Lovblom LE, Farooqi MA, Scarr D, Boulet G, Hertz P, Wu T, Halpern EM, Ngo M, Ng E, Orszag A, Bril V, Perkins BA. Reproducibility of In Vivo Corneal Confocal Microscopy Using an Automated Analysis Program for Detection of Diabetic Sensorimotor Polyneuropathy. *PLoS One* 2015; **10**: e0142309 [PMID: 26539984 DOI: 10.1371/journal.pone.0142309]

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## Diabetes mellitus and cognitive impairments

Elham Saedi, Mohammad Reza Gheini, Firoozeh Faiz, Mohammad Ali Arami

Elham Saedi, Amir Alam Research Center, Department of Internal Medicine, Division of Neurology, Amir Alam Hospital, Tehran University of Medical Sciences, Tehran 1416753955, Iran

Mohammad Reza Gheini, Department of Neurology, Sina Hospital, Tehran University of Medical Sciences, Tehran 1416753955, Iran

Firoozeh Faiz, Department of Internal Medicine, Division of Endocrinology, Amir Alam Hospital, Tehran University of Medical Sciences, Tehran 1416753955, Iran

Mohammad Ali Arami, Department of Neurology, Milad General Hospital, Tehran 1449614531, Iran

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**Correspondence to:** Mohammad Ali Arami, MD, Department of Neurology, Milad General Hospital, Hemmat Highway, Tehran 1449614531, Iran. [arami\\_ma@yahoo.com](mailto:arami_ma@yahoo.com)  
**Telephone:** +98-912-1571656  
**Fax:** +98-216-6760245

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### Abstract

There is strong evidence that diabetes mellitus increases the risk of cognitive impairment and dementia. Insulin signaling dysregulation and small vessel disease in the base of diabetes may be important contributing factors in Alzheimer's disease and vascular dementia pathogenesis, respectively. Optimal glycemic control in type 1 diabetes and identification of diabetic risk factors and prophylactic approach in type 2 diabetes are very important in the prevention of cognitive complications. In addition, hypoglycemic attacks in children and elderly should be avoided. Anti-diabetic medications especially Insulin may have a role in the management of cognitive dysfunction and dementia but further investigation is needed to validate these findings.

**Key words:** Alzheimer's disease; Cognitive disorders; Dementia; Diabetes; Insulin

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**Core tip:** Diabetes mellitus increases the risk of cognitive impairment and dementia. Impairment of insulin signaling is a critically important factor and may be the cornerstone of the development of these cognitive sequences regardless of diabetic status. Therefore, anti-diabetic medications especially insulin therapy may have a significant role in the management of various cognitive and mental dysfunctions.

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### INTRODUCTION

Type 2 diabetes mellitus (T2DM) is one of the most common diseases whose prevalence is on the rise. It

is believed that within the next 30 years, the number of diabetic patients will double in comparison to the year 2000<sup>[1]</sup>. On the other hand, diabetes is amongst the diseases with higher complications (perhaps even the highest) and these complications lower the quality of life in patients significantly<sup>[2-4]</sup>. Diabetes is a systemic disease as it affects various body systems to some extent. For instance, diabetes can disrupt proper function in cardiovascular, gastrointestinal, immune and nervous systems. The functional impairment of peripheral nervous system can lead to diabetic foot and in worst cases to amputation and hence physical disability. Involvement of retina [diabetic retinopathy (DR)] can lead to loss of vision and blindness.

Adverse effects of diabetes on cognitive system and memory disorders have been noticed by researchers for a long time<sup>[2-4]</sup>. Equally, dementia is one of the most disabling public health problems. It affects the quality of life of demented patients and their caregivers. It also imposes a huge economic burden on countries. Therefore, identification of risk factors of dementia and the control of those factors is with utmost importance.

This review discusses the association between diabetes and the risk of cognitive impairment with more clinical aspects. Therefore, possible underlying mechanisms of cognitive impairment in diabetic patients will be discussed, and the effect of various treatments on prophylaxis and improvement of mental dysfunction will be reviewed.

## OVERVIEW OF MEMORY AND COGNITION

Cognition is defined as "the mental action or process of acquiring knowledge and understanding through thought, experience, and the senses"<sup>[5]</sup>.

Memory is the retention, recording, and process of retrieving knowledge. All knowledge gained from experience such as known facts, remembered events, gained and applied skills would be considered as memory<sup>[6]</sup>. Memory can be categorized into declarative and non-declarative memory. Declarative memory mostly corresponds to the learning and recalling new facts, events, and materials. Non-declarative memory refers to the many forms of memories that are reflective or incidental<sup>[6]</sup>.

The "brain working memory" is defined as the ability to keep record of many bits of information at the same time and the recall of this information immediately if needed for subsequent thoughts<sup>[7]</sup>. When working memory is damaged, a wide range of cognition impairments occur and the patient will not be able to appropriately use his/her own information for thinking in different situations<sup>[6]</sup>.

The majority of advanced cortical functions arise from association cortex. The main association areas are: (1) the parieto-occipitotemporal association area; (2) the prefrontal association area; and (3) the limbic

association area<sup>[7]</sup>.

Our knowledge about the mechanisms of thinking and remembering is little. It seems that each thought arises from simultaneous activation of many parts of the different areas in the brain such as cerebral cortex, limbic system, thalamus and reticular formation of the brainstem. The memory is the result of some events in the synaptic transmission by changing its basic sensitivity<sup>[7]</sup>.

Constant neural activity that arises from traveling nerve signals to a temporary memory trace can create a "short term memory". A temporary chemical or physical synaptic change that lasts for a few minutes up to several weeks makes an "intermediate long term memory". Structural alterations in synapses occur when a "long term memory" is created and can be used weeks to years later<sup>[7]</sup>. The hippocampus and, to a lesser degree, the thalamus are responsible for deciding which thoughts are important enough to be saved as memories<sup>[7]</sup>.

It is possible to acquire information about the patient's cognitive, behavioral, linguistic, and executive functioning, and memory through Neuropsychological tests. These data can be used in the diagnosis of cognitive disorders and for localization of the abnormality in the brain, as well as, the assessment of therapeutic effects of any treatment modality on the cognitive dysfunction. Neurocognitive domains and some examples for their assessment are categorized in the Table 1<sup>[8,9]</sup>.

Neuropsychological evaluation measures the cognitive abilities in the patient quantitatively, and its results must be interpreted in the setting of the patient's: Age, education, gender, and cultural background. In addition, reliability, validity, sensitivity, and specificity of these tests are important aspects that should be considered.

## ETIOLOGY OF COGNITIVE DISORDERS

Dementia and cognitive dysfunction have many causes. Alzheimer's disease (AD) and other degenerative diseases, vascular dementia, alcohol consumption, and certain drug abuse are some of these etiologies. Additional disorders that can cause memory loss and other cognitive impairments are listed in the Table 2<sup>[9]</sup>.

## ASSOCIATION BETWEEN DIABETES AND COGNITIVE DECLINE

Cognitive dysfunction with its wide range, from mild cognitive impairment (MCI) through dementia, is one of the chronic complications of diabetes mellitus<sup>[10]</sup>. Both diabetes and cognitive impairment occur more commonly at older age. There is strong evidence that T2D increases the risk of dementia in the form of multi-infarct dementia, AD and mixed type dementia. There are some close associations between diabetes and vascular dementia of above 100%-160% compared to



**Table 1** Neurocognitive domains and some examples for their assessment<sup>[8,9]</sup>

Cognitive domain	Examples of assessments
Complex attention (sustained attention, divided attention, selective attention, processing speed)	Sustained attention: Maintenance of attention over time Selective attention: Maintenance of attention despite competing stimuli and/or distractors Divided attention: Attending to two tasks within the same time period Processing speed can be quantified on any task by timing it
Executive function (planning, decision making, working memory, mental flexibility)	Planning: Ability to find the exit to a maze; interpret a sequential picture Decision making: Performance of tasks that assess process of deciding in the face of competing alternatives (e.g., simulated gambling) Working memory: Ability to hold information for a brief period and to manipulate it (e.g., adding up a list of numbers or repeating a series of numbers or words backward) Mental/cognitive flexibility: Ability to shift between two concepts, tasks, or response rules
Learning and memory [immediate memory, recent memory (including free recall, cued recall, and recognition memory), very-long-term memory (semantic, autobiographical), implicit learning]	Immediate memory span: Ability to repeat a list of words or digits. Note: Immediate memory sometimes subsumed under "working memory" (see "Executive Function") Recent memory: Assesses the process of encoding new information (e.g., word lists, a short story, or diagrams) Free recall (the person is asked to recall as many words, diagrams, or elements of a story as possible) Cued recall (examiner aids recall by providing semantic cues such as "list all the food items on the list") Recognition memory (examiner asks about specific items, e.g., "Was 'apple' on the list?") Semantic memory (memory for facts) Autobiographical memory (memory for personal events or people) Implicit (procedural) learning (unconscious learning of skill)
Language [expressive language (including naming, word-finding, fluency, and grammar and syntax) and receptive language]	Expressive language: Confrontational naming (identification of objects or pictures) Fluency [e.g., name as many items as possible in a semantic (e.g., animals) or phonemic (e.g., words starting with "f") category in 1 min] Grammar and syntax (e.g., omission or incorrect use of articles, prepositions, auxiliary verbs) Receptive language: Comprehension, performance of actions/activities according to verbal command Visual perception: Line bisection tasks can be used to detect basic visual defect or intentional neglect
Perceptual-motor (includes abilities subsumed under the terms visual perception, visuomotor, perceptual-motor, praxis, and gnosis)	Visuoconstructional: Assembly of items requiring hand-eye coordination, such as drawing, copying, and block assembly Perceptual-motor: Integrating perception with purposeful movement (e.g., rapidly inserting pegs into a slotted board) Praxis: Integrity of learned movements, such as ability to imitate gestures (wave goodbye) or pantomime use of objects to command ("show me how you would use a hammer") Gnosis: Perceptual integrity of awareness and recognition, such as recognition of faces and colors
Social cognition (recognition of emotions, theory of mind)	Recognition of emotions: Identification of emotion in images of faces representing a variety of both positive and negative emotions Theory of mind: Ability to consider another person's mental state (thoughts, desires, intentions)

AD which is about 45% to 90%<sup>[10]</sup>. The long-term risk of dementia increases in patients with diabetes by a factor of two<sup>[11]</sup>. T2D also increases the risk of progression of MCI to dementia<sup>[11]</sup>. Even in pre-diabetic state; there is an increased risk of AD and dementia which are not related to the future development of diabetes<sup>[10]</sup>. About 80% of people with AD may have diabetes or impaired fasting glucose<sup>[12]</sup>. There is a faster deterioration of cognition in diabetic patients rather than non-diabetic elderly ones<sup>[13]</sup>. Diabetes is associated with 1.5-2 fold increased risk of cerebrovascular accidents<sup>[14]</sup> and the relative risk of stroke increases 1.15 (95%CI: 1.08-1.23) for every 1% increase in HbA1C<sup>[15]</sup>.

In recent years, the relation of diabetes to memory disorders has been well established. In 2011, Wessels *et al.*<sup>[16]</sup> published results of their comprehensive prospective study on a large sample size from 1992 to 2007. Patients in this cohort were examined at baseline and five follow-up assessments throughout the 15 years of study. During each evaluation, participants were given the Community Screening Interview for Dementia as part of a home visit. They followed up 1702 subjects and showed that diabetes reduced their cognitive capabilities

via cardiovascular disruption<sup>[16]</sup>. The results of the Edinburgh Type 2 Diabetes Study that was conducted for evaluation of this correlation were published in 2013. At baseline, any clinical and subclinical macrovascular diseases including cardiovascular event history, carotid intima-media thickness, ankle brachial index, and serum N-terminal probrain natriuretic peptide (NT-proBNP) were evaluated. Seven neuropsychological tests were also done at baseline, and after 4 years. They found that stroke and subclinical markers of cardiovascular and atherosclerosis are associated with cognitive decline in older patients with type 2 diabetes (T2D)<sup>[17]</sup>.

Recent research collaboration between Mayo Clinic and Shanghai was reported in 2015. In this study, involving a considerable number of patients, the effect of diabetes on the cognitive function of patients was strongly evident. This was, of course, irrespective of patients' gender, age and possible cardiovascular risk factors<sup>[18]</sup>.

In one study, the relationship between T2D and cognitive impairment had been evaluated and the subjects with diabetes had lower MMSE score than those without diabetes ( $P < 0.01$ )<sup>[19]</sup>. Diabetes was

**Table 2** Memory loss and cognitive impairment etiology<sup>[9]</sup>

Degenerative disorders including Alzheimer's disease
Vascular dementia
Depression and anxiety
Medication side effects
Disturbed sleep
Hormones
Metabolic disorders
Diabetes
Alcohol abuse
Lyme disease
Hippocampal sclerosis
Subdual and epidural hematomas
Vitamin B12 deficiency
Seizures
HIV associated neurocognitive disorder
Hashimoto's encephalopathy

HIV: Human immunodeficiency virus.

also associated with increased odds of cognitive decline as determined by MMSE scores [odds ratio (OR), 1.9; 95%CI: 1.01-3.6]. Also, a statistically significant correlation between the duration of the disease and cognitive dysfunction was observed ( $P = 0.001$ ). The same correlation was also found for the quality of diabetes control ( $P = 0.002$ ).

In a different study that was carried out on 4206 subjects by Qiu *et al.*<sup>[20]</sup>, they investigated whether and the extent to which vascular and degenerative lesions in the brain mediate the association of diabetes with poor cognitive performance. They assessed cortical and subcortical infarcts and higher white matter lesion volume. They also evaluated neurodegenerative processes on magnetic resonance images. The results of this cross-sectional study showed that diabetic patients' speed in processing and executive functions was markedly lower than others. However, their memory function score was not any better either<sup>[20]</sup>.

The role of diabetes in neurodegeneration has been confirmed by neuroimaging and neuropathological studies. MRI studies have shown that T2D is strongly associated with brain atrophy<sup>[21]</sup>. The rate of global brain atrophy in T2D is up to 3 times faster than in normal aging<sup>[22,23]</sup>.

## SPECIFIC EFFECTS OF T1D AND T2D ON COGNITION

Diabetes Mellitus is related to 40% higher rate of MCI; both amnesic and non-amnesic<sup>[24]</sup>. This is especially true when diabetes starts before the age of 65, or when the disease is more than 10 years. Treatment with insulin and the presence of diabetes complications such as retinopathy are other risk factors<sup>[25,26]</sup>.

In children, the relationship between T1D and cognitive disorders is also reported<sup>[27]</sup>. Cognitive flexibility, visual perception, psychomotor speed, and attention are the main domains which are mostly affected early (on within 2 years in T1D), among which

the mental slowing is the principal deficiency. Learning and memory function seem to be intact even in a prolonged hyperglycemia in T1D<sup>[25]</sup>. Young age is an important risk factor in developing cognitive deficits in T1D. It seems that children whose disease is diagnosed under the age of 7 are at a greater risk for more severe cognitive dysfunction<sup>[28]</sup>.

Single-photon emission tomography in diabetic patients shows an abnormality in many brain regions, which correlate especially with diabetic microvascular complications and poor glycemic control in T1D. However, there is no strong evidence to support the importance of brain perfusion abnormalities in the development of cognitive dysfunction in T1D<sup>[29]</sup>.

In both types of diabetes, neural slowing, cortical atrophy and microstructural abnormalities in white matter are prominent<sup>[24]</sup>.

The effect of diabetes on patients' mood and temper has also been investigated. In a recent article by Ho *et al.*<sup>[30]</sup>, they have pointed out the effects of diabetes on hippocampus neurogenesis and depression and the resulting cognitive.

## DR AND COGNITIVE IMPAIRMENT

It has been shown that there is an association between DR and cognitive impairment. According to some studies, the vascular complications of diabetes such as retinopathy are the most important predictors for the cognitive decline. Based on the similarity in anatomy, physiology, and embryology of cerebral and retinal small vessels, this association is particularly interesting<sup>[31]</sup>.

In a systematic review which analyzed three studies, it has been proven a near three fold increased risk of cognitive impairment in patients with DR. However; the association between the severity of DR and cognitive decline was not clearly demonstrated. Only one study showed that the men with more severe cognitive impairment had greater degree of retinal involvement. The recent memory and the verbal learning were the most defective cognitive domains in these studies<sup>[32]</sup>.

Some studies have reported an association between cognitive impairment and general (not diabetic) retinopathy independent of other cardiovascular risk factors but underlying etiology has not been clearly identified<sup>[33,34]</sup>. The higher prevalence of cognitive impairment even in those with non-DR provides some clues to investigate the underlying mechanism for this association in wider metabolic abnormalities (hypertension, dyslipidemia, and inflammatory stress) rather than a pure glucotoxic effect<sup>[32]</sup>.

In a longitudinal study from using Action to Control Cardiovascular Risk in Diabetes (ACCORD) data, the association between DR and cognitive impairment in T2M was confirmed. This study showed that cognitive dysfunction was a predictable consequence of DR. In the ACCORD data, the patients with DR had lower Mini-Mental State Examination (MMSE) score<sup>[35]</sup>.

In one cohort study by Crosby-Nwaobi *et al.*<sup>[36]</sup>, they

compared patients with Proliferative Diabetic Retinopathy with patients with Non Proliferative Diabetic Retinopathy or no retinopathy. They found that there is an inverse relationship between the severity of DR and the severity of cognitive impairments: Those with no or mild form of DR had more deficits in attention/orientation, language, memory, and visuospatial ability fields in comparison with patients with severe DR. However; their study showed that cognitive impairment was more prominent in those with mild retinopathy than those without retinopathy<sup>[36]</sup>.

## BRAIN IMAGING IN DIABETES

Brain imaging can be an important tool to clarify the underlying pathogenesis for cognitive impairments in diabetic patients. Some researchers have been reported both focal and global cerebral changes<sup>[37]</sup>.

Slight brain structural abnormalities have been reported in T1D<sup>[25,38]</sup>. A study showed that the gray matter density of patients with T1D was less than the control group and this finding correlated with severe hypoglycemic attacks and higher HbA1c levels. This assessment was performed with voxel-based morphometry - a well-known quantitative MRI technique<sup>[25,38]</sup>.

The direction of water diffusion in tissues is measured by using diffusion tensor imaging (DTI) that is an index for the integrity of white matter<sup>[25]</sup>. DTI shows microstructural abnormalities particularly in the optic radiations and posterior corona radiata in T1D patients. These findings correlate with longstanding diabetes and high concentrations of HbA1c<sup>[39]</sup>. These abnormalities may be the underlying pathogenesis in the mental slowing that is the main cognitive problem in T1D<sup>[40]</sup>. DTI Technique will be a good research tool for future studies in this setting.

There is a relationship between T2D and lacunar infarcts/cerebral Atrophy. This association between T2D and white matter lesions is less clear<sup>[37]</sup>. It was reported that hippocampal atrophy is a consistent neuroimaging finding in patients with T2D<sup>[41]</sup>, but a relatively recent study that evaluated the data from one cohort study and two case control studies, concluded that these patients did not have any specific vulnerability to hippocampal atrophy. Nevertheless; they have greater global brain atrophy compared to controls<sup>[42]</sup>.

## DIABETES MELLITUS, AD AND INSULIN ROLE

T2D is a condition in that elevated blood glucose levels is resulted from increased glucose production by liver, reduced insulin production by pancreas and "insulin resistance" in which insulin responsiveness is decreased due to abnormal expression of the insulin receptors<sup>[43]</sup>.

The idea that AD is a metabolic disease in which brain glucose utilization is impaired is supported by some evidences. Conversely; amyloid precursor protein

(APP) and amyloid- $\beta$ -peptide ( $A\beta$ ) have been shown to induce mitochondrial activity defects and increase oxidative stresses that are able to impair key players of the glucose metabolic pathway<sup>[44,45]</sup>.

The prevalence of AD may be higher in patients with diabetes; however, the ORs are lower than those for vascular dementia<sup>[46]</sup>. In recent years; there are a number of studies that show a connection (*via* comparing pathologic samples) between T2D and AD. Scientists consider a key role for oxidative stress in development of AD in patients with diabetes Mellitus<sup>[43]</sup>. Diabetes Mellitus contributes to AD development by favoring tau hyperphosphorylation, accumulation of  $A\beta$ , increased oxidative stress and oxidative damage and mitochondrial dysfunction<sup>[44]</sup>. In this regard, the analysis of oxidation and damage of protein belonging to metabolic pathways (glucose metabolism) might be of interest in understanding the potential molecular mechanisms targeted by oxidative stress that trigger common features between T2D and AD. Different studies have shown that insulin resistance and reduced activation of insulin receptors with decreased neuronal plasticity mechanisms and survival are the main abnormalities in AD brain<sup>[43,47-49]</sup>. Figure 1 illustrated some aspects of this mechanism<sup>[43]</sup>.

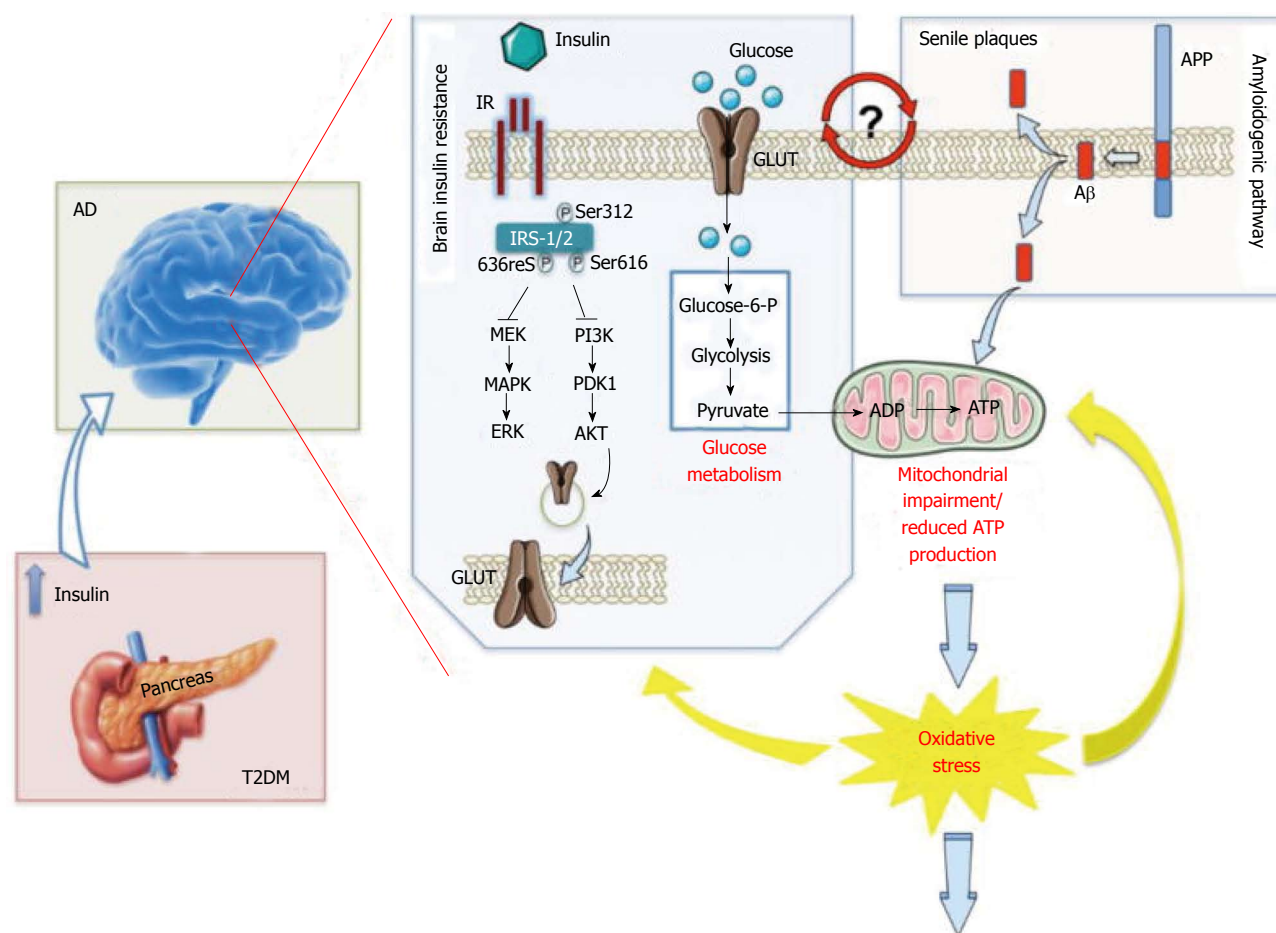
T2D is a heterogeneous disorder that is accompanied with numerous comorbidities like hypertension and dyslipidemia, where each has the same adverse effects on the cognitive function<sup>[50]</sup>. In addition, other insulin resistance situations including obesity and metabolic syndrome are associated with a wide range of cognitive dysfunction and progression of AD<sup>[25,51]</sup>.

Long term effects of insulin resistance consist of hypertension, malignancy and cardiovascular disease. It has been shown that insulin resistance has a negative correlation with verbal cognitive performance<sup>[25]</sup>.

Thus, insulin resistance seems to be the fundamental feature that links T2DM to the future development of AD. The biochemical and molecular changes in AD is similar to the effects of NASH (nonalcoholic steatohepatitis) on the liver and T2D on the skeletal muscles<sup>[52]</sup>. Long term outcomes of insulin resistance include cellular energy defect, high plasma lipids and hypertension<sup>[52]</sup>. In Addition, chronic hyperinsulinemia predicts later development of T2M<sup>[53]</sup>. Insulin resistance is also a definite predictor of serious conditions such as cerebrovascular and cardiovascular diseases, hypertension, and malignancy<sup>[52]</sup>. Hyperinsulinemia is linked to some other diseases with different primary target organs include: Obesity, nonalcoholic fatty liver disease, metabolic syndrome, polycystic ovarian disease, age-related macular degeneration. Overlap among these diseases often occurs and its rate is increasing with obesity epidemics<sup>[52]</sup>.

## INSULIN SIGNALING

There is significant amount of evidence demonstrating that dysregulation of insulin is a key element in trig-



**Figure 1** Increased oxidative stress level as a central event driving insulin resistance in Alzheimer's disease brain<sup>[43]</sup>. Persistently high levels of circulating insulin [as observed in the first phase of type 2 diabetes mellitus (T2DM)] may exert a negative influence on memory and other cognitive functions by down regulation of insulin receptors (IR) at the blood brain barrier and consequent reduced insulin transport into the brain [as observed in Alzheimer's disease (AD)], thus leading to insulin resistance. From a molecular point of view, the lack of interaction between insulin and IR is associated with an increase of the inhibitory phosphorylation on insulin receptor substrate-1/2 (IRS1/2) on Ser312, 616 and 636, which, in turn, negatively impacts on the two main arms of insulin-mediated signaling cascade: The PI3K and the MAPK pathways, both involved in the maintenance of synaptic plasticity and cell stress response. Furthermore, turning off insulin signaling results in impaired glucose transport (reduced translocation of the glucose transporter at the plasma membrane) and metabolism thus promoting an alteration of mitochondrial processes involved in energy production. In turn, impairment of mitochondria functions leads to a vicious circle in which reduced energy production is associated with an increase of reactive oxygen and nitrogen species (ROS and RNS) responsible for the oxidative/nitrosative damage of mitochondria as well as other cellular components. In addition, increased A $\beta$  production and accumulation, which represents a key feature of AD pathology, also promotes mitochondrial impairment. Moreover, insulin resistance-associated impairments in glucose uptake and utilization are associated with increased endoplasmic reticulum (ER) stress, which deregulate lipid metabolism, causing accumulation of toxic lipids in the brain. All these events contribute to the increased oxidative stress levels responsible of neurodegeneration observed in AD brain. Although insulin resistance and A $\beta$  production can be considered leading causes of the rise of oxidative stress, this latter, in turn, promotes IRS-1/2 Ser-312, -616 and -636 phosphorylation as well as the oxidative damage of protein involved in glycolysis, the Krebs cycle and ATP synthesis that are crucial events in the reduction of glucose metabolism and thus insulin resistance. Finally, because insulin resistance is associated with increased A $\beta$  production and A $\beta$  production is postulated to be responsible for the onset of insulin resistance, it remains to be clarified whether insulin resistance is a cause, consequence, or compensatory response to A $\beta$ -induced neurodegeneration. ADP: Adenosine diphosphate; APP:  $\beta$ -Amyloid precursor protein; ATP: Adenosine triphosphate; AKT: Akt also known as protein kinase B (PKB); ERK: Extracellular signal-regulated kinase; GLUT: Glucose transporter; MAPK: Mitogen-activated protein kinase; MEK: MAPK/Erk kinase; PDK1: 3-phosphoinositide-dependent protein kinase 1; PI3K: Phosphoinositide 3 kinase.

gering of neurodegeneration in T2D. Insulin binds to a specific receptor at blood brain barrier and transport into the CNS. It is shown that an acute increase in serum insulin levels is associated with an increase in CSF and intracellular insulin levels<sup>[54,55]</sup>. Also, it is reported that chronic hyperinsulinemia is associated with downregulation of insulin receptor at blood brain barrier<sup>[54]</sup> which decrease brain insulin levels and consequently trigger or accelerate the process of neural aging and neurodegeneration<sup>[54,55]</sup>. Studies have shown that hyperinsulinemia causes an increase in A $\beta$  levels

as well as the inflammatory agents<sup>[56]</sup> and alter the metabolism of amyloid in the brain<sup>[46-57]</sup>.

It seems, insulin has a neurotropic role in the brain. Insulin accomplishes this role by binding to insulin receptors on the cell surface. It is interesting that most of insulin receptors in the brain are on the surface of the cells, located in anatomical regions that are involved in memory formation. So it is postulated that insulin might play an important role in the memory system<sup>[54]</sup>.

Insulin activates secondary messengers after binding to receptors. The most important of these secondary



messengers are phosphatidylinositol-3-kinase and Akt<sup>[54]</sup>. Activation of Akt causes inhibition of GSK-3 $\beta$ , which is an important kinase that phosphorylates tau. In fact, it is shown that under normal conditions, insulin inhibits tau phosphorylation and tau fibril production and low CSF insulin levels are associated with an increased neurofibrillary tangles<sup>[54,55]</sup>. Neurofibrillary tangles load is the best pathological marker of severity of dementia in AD.

Additionally, it's known that A $\beta$  protein is degraded by several enzymes. The most important of these enzymes are neprilysin and insulin-degrading enzyme (IDE)<sup>[58]</sup>. Both insulin and A $\beta$  protein can bind to IDE and it is shown that insulin has higher affinity to IDE<sup>[54]</sup>. It is shown that hyperinsulinemia might inhibits peripheral degradation of A $\beta$  protein<sup>[59]</sup>. High level of A $\beta$  protein can lead to an increase transport of this protein across blood brain barrier, which is shown to be associated with an increased production of senile plaques in the brain<sup>[59]</sup>.

In conclusion, it is hypothesized that serum hyperinsulinemia is associated with lower level of insulin and higher level of A $\beta$  protein in the brain, resulting in more neurofibrillary tangles, senile plaques, and possibly with impaired cognitive state.

## IS AD A TYPE OF DIABETES MELLITUS?

AD is considered as type 3 diabetes by some investigators because the corner stone of pathogenesis of abnormalities in AD has strong similarity with T1D and T2D. Like T1D, insulin deficiency is a part of underlying mechanisms in AD and like T2D, AD is associated with insulin resistance in early stage of development<sup>[52,60,61]</sup>. Consequently, AD can be considered as the brain form of diabetes<sup>[52]</sup>.

Nevertheless; Talbot *et al*<sup>[62]</sup>, reported some evidence that considering AD as a type of diabetes is not completely true due to the following: First, hyperglycemia but not insulin resistance is the main key diagnostic feature of diabetes, and CSF glucose is not elevated in AD patients. Second, decreased glucose metabolism in the brain AD cases is not a direct consequence of brain insulin resistance. Instead of that, postsynaptic neurotransmission changes due to reduced insulin signaling are responsible for abnormal glucose metabolism in the AD brain. Third, brain insulin deficiency in the AD patients has not been established from the review of different studies, and only some of them have shown this decrement<sup>[62]</sup>.

## OTHER MECHANISM OF COGNITIVE IMPAIRMENT IN DIABETES MELLITUS

### Vascular etiology

T2D is a risk factor for atherosclerosis and small vessel disease, so it clearly increases the risk of multi-infarct dementia and mixed type dementia. Other risk factors of vascular disease contribute to the development of

dementia in patients with T2D, probably by vascular involvement. It has been shown that in patients with T2D, presence of hypertension, signs of microvascular diseases such as lacuna, DR and microalbuminuria or macrovascular complications such as cerebral infarcts increase the risks of dementia<sup>[54,63]</sup>.

### Chronic inflammation

Chronic inflammation is present in many patients with diabetes and insulin resistance is associated with increased levels of inflammatory cytokines, which elevated levels of inflammatory cytokines are associated with the worsening of the cognition in patients with diabetes<sup>[46,64]</sup>.

### Genetic

Brain changes and reductions in cognitive scores are most pronounced in patients with diabetes who have the Apo E epsilon 4 allele. The genetic factors contribute to dementia in T2D<sup>[65]</sup>.

## THERAPEUTIC APPROACHES

According to the long term prospective studies, good control of diabetes is beneficial in the reduction of cognitive decline in T1D<sup>[25,29]</sup>, but the effect of this approach in T2D is controversial<sup>[66-70]</sup>. In one cohort study, there was a greater decline in cognitive impairment in patients on anti-diabetic medications and combination therapy was more effective than monotherapy<sup>[69]</sup>.

One substudy of the ACCORD trial that followed up a large number of diabetic patients for 40 mo, showed no benefits from aggressive glucose control on the cognitive function<sup>[70]</sup>. In addition; three trials showed that intensive glycemic control has no benefit on the macrovascular events in T2D<sup>[66-68]</sup>.

Association between cognitive decline and hypoglycemic attacks has been studied in some trials but the results are different. Overall, it seems that it is not a risk factor in T2D in carefully managed follow up studies. However, the prevention from hypoglycemia in the elderly is necessary, because it can cause more severe organic brain damage due to pre-existing atherosclerosis<sup>[71]</sup>. Also, it is true that, hypoglycemia may be a risk factor in children with diagnosed T1D within the first few years of life<sup>[25]</sup>. However, recurrent severe hypoglycemia is a significant preventable risk factor in these age groups and individualization of treatment, especially in the elderly, has a potential role in preventing hypoglycemia and consequently cognitive decline. While diabetes *per se* has a major impact on the elderly, the medications and the risk of hypoglycemia prevent optimization of glycemic treatment<sup>[72]</sup>.

In one study, daily acute glucose fluctuation was an independent factor for cognitive dysfunction in T2D<sup>[73]</sup>. In another study, there was an association between cognitive impairment and postprandial hyperglycemia.

There was a greater decline in cognitive impairment after adjusting for postprandial hyperglycemia<sup>[74]</sup>.

The thiazolidinedione classes of anti-diabetic medications are insulin sensitizers that work by making the cells more sensitive to insulin. Most of the research has focused on the effect of thiazolidinedione on improvement of cognitive function. The findings suggest that there is continuous beneficial effect of insulin sensitizers on cognition. Its effect is more pronounced on neuron action by reduction of apoptosis, protecting neurons from oxidative stress and reducing plaque formation and inflammation in mice brain models. Despite these findings, clinical trials in human are disappointing<sup>[10]</sup>.

Insulin action has a contributing factor in cognitive function. Both insulin resistance and hyperinsulinemia are associated with cognitive impairment<sup>[71]</sup>. Excessive hyperinsulinemia exacerbates inflammation. Hyperinsulinemia enhances neurotic plaque formation<sup>[75]</sup>. Insulin secretion reduction is also associated with the onset of AD. Insulin definitively is connected with AD pathology and vascular dementia<sup>[76]</sup>.

Intranasal insulin was effective in the improvement of memory function in memory impaired adults, in some studies<sup>[9]</sup>. Indeed, about 50% of all adults older than 60 years, even in the absence of diabetes, are insulin resistant<sup>[56]</sup>. It seems insulin puts its effect on cognitive function by modulation in aggregation of APP metabolites like beta amyloid peptide in neurotic plaques. On the other hand, factors associated with insulin resistance are suggested to be important in pathogenesis of AD. As it has been shown, Apo E negative patients are less sensitive to insulin which makes them in need for a higher level of insulin to facilitate an effective memory function in AD<sup>[77]</sup>.

To date, there are few clinical data on the efficacy of metformin in AD and because of conflicting results regarding the effect of metformin in the improvement or deterioration of cognitive impairment, it needs to be clarified by a clinical placebo- controlled trial<sup>[78]</sup>.

Other than hyperglycemia, midlife hypertension, midlife obesity, smoking, depression, and physical inactivity are attributable risk factors in AD and a 25% reduction in all of these factors could reduce the number of dementia by up to 3 million<sup>[79]</sup>. Large scale studies have shown that: Good control on blood pressure and lipid profile as well as glucose control will prevent vascular disease progression<sup>[80]</sup>.

## CONCLUSION

There is strong evidence that diabetes increases the risk of cognitive impairment and dementia. Insulin signaling dysregulation may be an important contributing factor in AD pathogenesis. In addition, diabetes is a risk factor for atherosclerosis and small vessel disease. It clearly increases the risk of vascular dementia. Good control of diabetes is beneficial in the reduction of cognitive decline in T1D, but the effect of this approach in improving

cognitive outcomes in T2D is weak. Therefore; optimal glycemic control in T1D, identification of diabetic risk factors, and prophylactic approach in T2D are very important in the Prevention of cognitive complications. Lifestyle intervention such as proper diet and physical activity is the most important approaches in this way.

As the brain dysfunction in AD could be the result of disturbance in glucose metabolism and its dysregulation regardless of the diabetic status, future research with focus on anti-diabetic medications may open a new horizon for the prevention and management of AD. In addition, due to similarity in molecular and biochemical base of T2M and AD, more investigations in the domain of insulin resistance spectrum disorders provide an opportunity to find novel treatment strategies. These new approaches will be based on the improvement in the understanding of the pathogenesis of these fundamentally related disorders.

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## REFERENCES

- 1 **Wild S**, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004; **27**: 1047-1053 [PMID: 15111519 DOI: 10.2337/diacare.27.5.1047]
- 2 **Ott A**, Stolk RP, van Harskamp F, Pols HA, Hofman A, Breteler MM. Diabetes mellitus and the risk of dementia: The Rotterdam Study. *Neurology* 1999; **53**: 1937-1942 [PMID: 10599761 DOI: 10.1212/WNL.53.9.1937]
- 3 **Leibson CL**, Rocca WA, Hanson VA, Cha R, Kokmen E, O'Brien PC, Palumbo PJ. Risk of dementia among persons with diabetes mellitus: a population-based cohort study. *Am J Epidemiol* 1997; **145**: 301-308 [PMID: 9054233]
- 4 **Curb JD**, Rodriguez BL, Abbott RD, Petrovitch H, Ross GW, Masaki KH, Foley D, Blanchette PL, Harris T, Chen R, White LR. Longitudinal association of vascular and Alzheimer's dementias, diabetes, and glucose tolerance. *Neurology* 1999; **52**: 971-975 [PMID: 10102414 DOI: 10.1212/WNL.52.5.971]
- 5 **Oxford Dictionaries**. Definition of cognition. [accessed 2016 Feb 4]. Available from: URL: <http://www.oxforddictionaries.com/definition/english/cognition>
- 6 **Brewer JB**, Gabrieli JDE, Preston AR, Vaidya CJ, Rosen AC. Memory. In: Goetz CG. Textbook of Clinical Neurology. 3rd ed. Saunders: Elsevier Inc., 2007: 63-77
- 7 **Hall JE**. Guyton and Hall text book of medical physiology. 12th ed. Saunders: Elsevier Inc., 2010: 714-727
- 8 **American Psychiatric Association**. Diagnostic and Statistical Manual of Mental Disorders. 5th ed. APA: Washington, DC, 2013: 593-595
- 9 **Budson AE**, Solomon PR. Memory Loss, Alzheimer's Disease, and Dementia: A Practical Guide for Clinicians. 2nd ed. Elsevier: Elsevier Inc., 2016: 145-154
- 10 **Kravitz E**, Schmeidler J, Schnaider Beeri M. Type 2 diabetes and cognitive compromise: potential roles of diabetes-related therapies.

- Endocrinol Metab Clin North Am* 2013; **42**: 489-501 [PMID: 24011882 DOI: 10.1016/j.ecl.2013.05.009]
- 11 **Biessels GJ**, Strachan MW, Visseren FL, Kappelle LJ, Whitmer RA. Dementia and cognitive decline in type 2 diabetes and prediabetic stages: towards targeted interventions. *Lancet Diabetes Endocrinol* 2014; **2**: 246-255 [PMID: 24622755 DOI: 10.1016/S2213-8587(13)70088-3]
  - 12 **Janson J**, Laedtke T, Parisi JE, O'Brien P, Petersen RC, Butler PC. Increased risk of type 2 diabetes in Alzheimer disease. *Diabetes* 2004; **53**: 474-481 [PMID: 14747300 DOI: 10.2337/diabetes.53.2.474]
  - 13 **Ravona-Springer R**, Luo X, Schmeidler J, Wysocki M, Lesser G, Rapp M, Dahlman K, Grossman H, Haroutunian V, Schnaider Beeri M. Diabetes is associated with increased rate of cognitive decline in questionably demented elderly. *Dement Geriatr Cogn Disord* 2010; **29**: 68-74 [PMID: 20130405 DOI: 10.1159/000265552]
  - 14 **Folsom AR**, Rasmussen ML, Chambless LE, Howard G, Cooper LS, Schmidt MI, Heiss G. Prospective associations of fasting insulin, body fat distribution, and diabetes with risk of ischemic stroke. The Atherosclerosis Risk in Communities (ARIC) Study Investigators. *Diabetes Care* 1999; **22**: 1077-1083 [PMID: 10388971 DOI: 10.2337/diacare.22.7.1077]
  - 15 **Selvin E**, Bolen S, Yeh HC, Wiley C, Wilson LM, Marinopoulos SS, Feldman L, Vassy J, Wilson R, Bass EB, Brancati FL. Cardiovascular outcomes in trials of oral diabetes medications: a systematic review. *Arch Intern Med* 2008; **168**: 2070-2080 [PMID: 18955635 DOI: 10.1001/archinte.168.19.2070]
  - 16 **Wessels AM**, Lane KA, Gao S, Hall KS, Unverzagt FW, Hendrie HC. Diabetes and cognitive decline in elderly African Americans: a 15-year follow-up study. *Alzheimers Dement* 2011; **7**: 418-424 [PMID: 21784353 DOI: 10.1016/j.jalz.2010.07.003]
  - 17 **Feinkohl I**, Keller M, Robertson CM, Morling JR, Williamson RM, Nee LD, McLachlan S, Sattar N, Welsh P, Reynolds RM, Russ TC, Deary IJ, Strachan MW, Price JF. Clinical and subclinical macrovascular disease as predictors of cognitive decline in older patients with type 2 diabetes: the Edinburgh Type 2 Diabetes Study. *Diabetes Care* 2013; **36**: 2779-2786 [PMID: 23579182 DOI: 10.2337/dc12-2241]
  - 18 **Zhao Q**, Roberts RO, Ding D, Cha R, Guo Q, Meng H, Luo J, Machulda MM, Shane Pankratz V, Wang B, Christianson TJ, Aakre JA, Knopman DS, Boeve BF, Hong Z, Petersen RC. Diabetes is Associated with Worse Executive Function in Both Eastern and Western Populations: Shanghai Aging Study and Mayo Clinic Study of Aging. *J Alzheimers Dis* 2015; **47**: 167-176 [PMID: 26402765 DOI: 10.3233/JAD-150073]
  - 19 **Ebady SA**, Arami MA, Shafigh MH. Investigation on the relationship between diabetes mellitus type 2 and cognitive impairment. *Diabetes Res Clin Pract* 2008; **82**: 305-309 [PMID: 18848366 DOI: 10.1016/j.diabres.2008.08.020]
  - 20 **Qiu C**, Sigurdsson S, Zhang Q, Jonsdottir MK, Kjartansson O, Eiriksdottir G, Garcia ME, Harris TB, van Buchem MA, Gudnason V, Launer LJ. Diabetes, markers of brain pathology and cognitive function: the Age, Gene/Environment Susceptibility-Reykjavik Study. *Ann Neurol* 2014; **75**: 138-146 [PMID: 24243491 DOI: 10.1002/ana.24063]
  - 21 **Moran C**, Phan TG, Chen J, Blizzard L, Beare R, Venn A, Münch G, Wood AG, Forbes J, Greenaway TM, Pearson S, Srikanth V. Brain atrophy in type 2 diabetes: regional distribution and influence on cognition. *Diabetes Care* 2013; **36**: 4036-4042 [PMID: 23939539 DOI: 10.2337/dc13-0143]
  - 22 **Kooistra M**, Geerlings MI, Mali WP, Vincken KL, van der Graaf Y, Biessels GJ. Diabetes mellitus and progression of vascular brain lesions and brain atrophy in patients with symptomatic atherosclerotic disease. The SMART-MR study. *J Neurol Sci* 2013; **332**: 69-74 [PMID: 23835088 DOI: 10.1016/j.jns.2013.06.019]
  - 23 **van Elderen SG**, de Roos A, de Craen AJ, Westendorp RG, Blauw GJ, Jukema JW, Bollen EL, Middeldijk HA, van Buchem MA, van der Grond J. Progression of brain atrophy and cognitive decline in diabetes mellitus: a 3-year follow-up. *Neurology* 2010; **75**: 997-1002 [PMID: 20837967 DOI: 10.1212/WNL.0b013e3181f25f06]
  - 24 **Luchsinger JA**, Reitz C, Patel B, Tang MX, Manly JJ, Mayeux R. Relation of diabetes to mild cognitive impairment. *Arch Neurol* 2007; **64**: 570-575 [PMID: 17420320 DOI: 10.1001/archneur.64.4.570]
  - 25 **McCrimmon RJ**, Ryan CM, Frier BM. Diabetes and cognitive dysfunction. *Lancet* 2012; **379**: 2291-2299 [PMID: 22683129 DOI: 10.1016/S0140-6736(12)60360-2]
  - 26 **Roberts RO**, Geda YE, Knopman DS, Christianson TJ, Pankratz VS, Boeve BF, Vella A, Rocca WA, Petersen RC. Association of duration and severity of diabetes mellitus with mild cognitive impairment. *Arch Neurol* 2008; **65**: 1066-1073 [PMID: 18695056 DOI: 10.1001/archneur.65.8.1066]
  - 27 **Cato MA**, Mauras N, Ambrosino J, Bondurant A, Conrad AL, Kollman C, Cheng P, Beck RW, Ruedy KJ, Aye T, Reiss AL, White NH, Hershey T. Cognitive functioning in young children with type 1 diabetes. *J Int Neuropsychol Soc* 2014; **20**: 238-247 [PMID: 24512675 DOI: 10.1017/S1355617713001434]
  - 28 **Ryan CM**. Diabetes and brain damage: more (or less) than meets the eye? *Diabetologia* 2006; **49**: 2229-2233 [PMID: 16917756 DOI: 10.1007/s00125-006-0392-3]
  - 29 **Jacobson AM**, Ryan CM, Cleary PA, Waberski BH, Weinger K, Musen G, Dahms W. Biomedical risk factors for decreased cognitive functioning in type 1 diabetes: an 18 year follow-up of the Diabetes Control and Complications Trial (DCCT) cohort. *Diabetologia* 2011; **54**: 245-255 [PMID: 20803190 DOI: 10.1007/s00125-010-1883-9]
  - 30 **Ho N**, Sommers MS, Lucki I. Effects of diabetes on hippocampal neurogenesis: links to cognition and depression. *Neurosci Biobehav Rev* 2013; **37**: 1346-1362 [PMID: 23680701 DOI: 10.1016/j.neubiorev.2013.03.010]
  - 31 **Patton N**, Aslam T, Macgillivray T, Pattie A, Deary IJ, Dhillon B. Retinal vascular image analysis as a potential screening tool for cerebrovascular disease: a rationale based on homology between cerebral and retinal microvasculatures. *J Anat* 2005; **206**: 319-348 [PMID: 15817102 DOI: 10.1111/j.1469-7580.2005.00395.x]
  - 32 **Crosby-Nwaobi R**, Sivaprasad S, Forbes A. A systematic review of the association of diabetic retinopathy and cognitive impairment in people with Type 2 diabetes. *Diabetes Res Clin Pract* 2012; **96**: 101-110 [PMID: 22154373 DOI: 10.1016/j.diabres.2011.11.010]
  - 33 **Lesage SR**, Mosley TH, Wong TY, Szklo M, Knopman D, Catellier DJ, Cole SR, Klein R, Coresh J, Coker LH, Sharrett AR. Retinal microvascular abnormalities and cognitive decline: the ARIC 14-year follow-up study. *Neurology* 2009; **73**: 862-868 [PMID: 19752453 DOI: 10.1212/WNL.0b013e3181b78436]
  - 34 **Liew G**, Mitchell P, Wong TY, Lindley RI, Cheung N, Kaushik S, Wang JJ. Retinal microvascular signs and cognitive impairment. *J Am Geriatr Soc* 2009; **57**: 1892-1896 [PMID: 19737331 DOI: 10.1111/j.1532-5415.2009.02459.x]
  - 35 **Hugenschmidt CE**, Lovato JF, Ambrosius WT, Bryan RN, Gerstein HC, Horowitz KR, Launer LJ, Lazar RM, Murray AM, Chew EY, Danis RP, Williamson JD, Miller ME, Ding J. The cross-sectional and longitudinal associations of diabetic retinopathy with cognitive function and brain MRI findings: the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial. *Diabetes Care* 2014; **37**: 3244-3252 [PMID: 25193529 DOI: 10.2337/dc14-0502]
  - 36 **Crosby-Nwaobi RR**, Sivaprasad S, Amiel S, Forbes A. The relationship between diabetic retinopathy and cognitive impairment. *Diabetes Care* 2013; **36**: 3177-3186 [PMID: 23633523 DOI: 10.2337/dc12-2141]
  - 37 **van Harten B**, de Leeuw FE, Weinstein HC, Scheltens P, Biessels GJ. Brain imaging in patients with diabetes: a systematic review. *Diabetes Care* 2006; **29**: 2539-2548 [PMID: 17065699 DOI: 10.2337/dc06-1637]
  - 38 **Musen G**, Lyoo IK, Sparks CR, Weinger K, Hwang J, Ryan CM, Jimerson DC, Hennen J, Renshaw PF, Jacobson AM. Effects of type 1 diabetes on gray matter density as measured by voxel-based morphometry. *Diabetes* 2006; **55**: 326-333 [PMID: 16443764 DOI: 10.2337/diabetes.55.02.06.db05-0520]
  - 39 **Kodl CT**, Franc DT, Rao JP, Anderson FS, Thomas W, Mueller BA, Lim KO, Seaquist ER. Diffusion tensor imaging identifies deficits in white matter microstructure in subjects with type 1 diabetes that



- correlate with reduced neurocognitive function. *Diabetes* 2008; **57**: 3083-3089 [PMID: 18694971 DOI: 10.2337/db08-0724]
- 40 **Franc DT**, Kodl CT, Mueller BA, Muetzel RL, Lim KO, Seaquist ER. High connectivity between reduced cortical thickness and disrupted white matter tracts in long-standing type 1 diabetes. *Diabetes* 2011; **60**: 315-319 [PMID: 20980455 DOI: 10.2337/db10-0598]
  - 41 **Gold SM**, Dziobek I, Sweat V, Tirsi A, Rogers K, Bruehl H, Tsui W, Richardson S, Javier E, Convit A. Hippocampal damage and memory impairments as possible early brain complications of type 2 diabetes. *Diabetologia* 2007; **50**: 711-719 [PMID: 17334649 DOI: 10.1007/s00125-007-0602-7]
  - 42 **Wisse LE**, de Bresser J, Geerlings MI, Reijmer YD, Portegies ML, Brundel M, Kappelle LJ, van der Graaf Y, Biessels GJ. Global brain atrophy but not hippocampal atrophy is related to type 2 diabetes. *J Neurol Sci* 2014; **344**: 32-36 [PMID: 24958596 DOI: 10.1016/j.jns.2014.06.008]
  - 43 **Butterfield DA**, Di Domenico F, Barone E. Elevated risk of type 2 diabetes for development of Alzheimer disease: a key role for oxidative stress in brain. *Biochim Biophys Acta* 2014; **1842**: 1693-1706 [PMID: 24949886 DOI: 10.1016/j.bbdis.2014.06.010]
  - 44 **Butterfield DA**. Oxidative stress in neurodegenerative disorders. *Antioxid Redox Signal* 2006; **8**: 1971-1973 [PMID: 17034342 DOI: 10.1089/ars.2006.8.197]
  - 45 **Markesbery WR**. Oxidative stress hypothesis in Alzheimer's disease. *Free Radic Biol Med* 1997; **23**: 134-147 [PMID: 9165306]
  - 46 **Meneilly GS**, Tessier DM. Diabetes, Dementia and Hypoglycemia. *Can J Diabetes* 2016; **40**: 73-76 [PMID: 26778684 DOI: 10.1016/j.cjcd.2015.09.006]
  - 47 **Rivera EJ**, Goldin A, Fulmer N, Tavares R, Wands JR, de la Monte SM. Insulin and insulin-like growth factor expression and function deteriorate with progression of Alzheimer's disease: link to brain reductions in acetylcholine. *J Alzheimers Dis* 2005; **8**: 247-268 [PMID: 16340083]
  - 48 **Steen E**, Terry BM, Rivera EJ, Cannon JL, Neely TR, Tavares R, Xu XJ, Wands JR, de la Monte SM. Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease--is this type 3 diabetes? *J Alzheimers Dis* 2005; **7**: 63-80 [PMID: 15750215]
  - 49 **Talbot K**, Wang HY. The nature, significance, and glucagon-like peptide-1 analog treatment of brain insulin resistance in Alzheimer's disease. *Alzheimers Dement* 2014; **10**: S12-S25 [PMID: 24529520 DOI: 10.1016/j.jalz.2013.12.007]
  - 50 **van den Berg E**, Kloppenborg RP, Kessels RP, Kappelle LJ, Biessels GJ. Type 2 diabetes mellitus, hypertension, dyslipidemia and obesity: A systematic comparison of their impact on cognition. *Biochim Biophys Acta* 2009; **1792**: 470-481 [PMID: 18848880 DOI: 10.1016/j.bbdis.2008.09.004]
  - 51 **Shanik MH**, Xu Y, Skrha J, Dankner R, Zick Y, Roth J. Insulin resistance and hyperinsulinemia: is hyperinsulinemia the cart or the horse? *Diabetes Care* 2008; **31** Suppl 2: S262-S268 [PMID: 18227495 DOI: 10.2337/dc08-s264]
  - 52 **de la Monte SM**. Relationships between diabetes and cognitive impairment. *Endocrinol Metab Clin North Am* 2014; **43**: 245-267 [PMID: 24582101 DOI: 10.1016/j.ecl.2013.09.006]
  - 53 **Dankner R**, Chetrit A, Shanik MH, Raz I, Roth J. Basal-state hyperinsulinemia in healthy normoglycemic adults is predictive of type 2 diabetes over a 24-year follow-up: a preliminary report. *Diabetes Care* 2009; **32**: 1464-1466 [PMID: 19435961 DOI: 10.2337/dc09-0153]
  - 54 **Verdile G**, Fuller SJ, Martins RN. The role of type 2 diabetes in neurodegeneration. *Neurobiol Dis* 2015; **84**: 22-38 [PMID: 25926349 DOI: 10.1016/j.nbd.2015.04.008]
  - 55 **Moreira PI**, Duarte AI, Santos MS, Rego AC, Oliveira CR. An integrative view of the role of oxidative stress, mitochondria and insulin in Alzheimer's disease. *J Alzheimers Dis* 2009; **16**: 741-761 [PMID: 19387110 DOI: 10.3233/JAD-2009-0972]
  - 56 **Craft S**. Insulin resistance and Alzheimer's disease pathogenesis: potential mechanisms and implications for treatment. *Curr Alzheimer Res* 2007; **4**: 147-152 [PMID: 17430239 DOI: 10.2174/156720507780362137]
  - 57 **Ninomiya T**. Diabetes mellitus and dementia. *Curr Diab Rep* 2014; **14**: 487 [PMID: 24623199 DOI: 10.1007/s11892-014-0487-z]
  - 58 **Nalivaeva NN**, Belyaev ND, Kerridge C, Turner AJ. Amyloid-clearing proteins and their epigenetic regulation as a therapeutic target in Alzheimer's disease. *Front Aging Neurosci* 2014; **6**: 235 [PMID: 25278875 DOI: 10.3389/fnagi.2014.00235]
  - 59 **Gasparini L**, Gouras GK, Wang R, Gross RS, Beal MF, Greengard P, Xu H. Stimulation of beta-amyloid precursor protein trafficking by insulin reduces intraneuronal beta-amyloid and requires mitogen-activated protein kinase signaling. *J Neurosci* 2001; **21**: 2561-2570 [PMID: 11306609]
  - 60 **Arab L**, Sadeghi R, Walker DG, Lue LF, Sabbagh MN. Consequences of Aberrant Insulin Regulation in the Brain: Can Treating Diabetes be Effective for Alzheimer's Disease. *Curr Neuropharmacol* 2011; **9**: 693-705 [PMID: 22654727 DOI: 10.2174/157015911798376334]
  - 61 **Wang X**, Zheng W, Xie JW, Wang T, Wang SL, Teng WP, Wang ZY. Insulin deficiency exacerbates cerebral amyloidosis and behavioral deficits in an Alzheimer transgenic mouse model. *Mol Neurodegener* 2010; **5**: 46 [PMID: 21044348 DOI: 10.1186/1750-1326-5-46]
  - 62 **Talbot K**, Wang HY, Kazi H, Han LY, Bakshi KP, Stucky A, Fuino RL, Kawaguchi KR, Samoyedny AJ, Wilson RS, Arvanitakis Z, Schneider JA, Wolf BA, Bennett DA, Trojanowski JQ, Arnold SE. Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline. *J Clin Invest* 2012; **122**: 1316-1338 [PMID: 22476197 DOI: 10.1172/JCI59903]
  - 63 **Saczynski JS**, Siggurdsson S, Jonsson PV, Eiriksdottir G, Olafsdottir E, Kjartansson O, Harris TB, van Buchem MA, Gudnason V, Launer LJ. Glycemic status and brain injury in older individuals: the age gene/environment susceptibility-Reykjavik study. *Diabetes Care* 2009; **32**: 1608-1613 [PMID: 19509008 DOI: 10.2337/dc08-2300]
  - 64 **Strachan MW**. R D Lawrence Lecture 2010. The brain as a target organ in Type 2 diabetes: exploring the links with cognitive impairment and dementia. *Diabet Med* 2011; **28**: 141-147 [PMID: 21219420 DOI: 10.1111/j.1464-5491.2010.03199.x]
  - 65 **Dore GA**, Elias MF, Robbins MA, Elias PK, Nagy Z. Presence of the APOE epsilon4 allele modifies the relationship between type 2 diabetes and cognitive performance: the Maine-Syracuse Study. *Diabetologia* 2009; **52**: 2551-2560 [PMID: 19693485 DOI: 10.1007/s00125-009-1497-2]
  - 66 **Gerstein HC**, Miller ME, Byington RP, Goff DC, Bigger JT, Buse JB, Cushman WC, Genuth S, Ismail-Beigi F, Grimm RH, Probstfield JL, Simons-Morton DG, Friedewald WT. Effects of intensive glucose lowering in type 2 diabetes. *N Engl J Med* 2008; **358**: 2545-2559 [PMID: 18539917 DOI: 10.1056/NEJMoa0802743]
  - 67 **Duckworth W**, Abairra C, Moritz T, Reda D, Emanuele N, Reaven PD, Zieve FJ, Marks J, Davis SN, Hayward R, Warren SR, Goldman S, McCarren M, Vitek ME, Henderson WG, Huang GD. Glucose control and vascular complications in veterans with type 2 diabetes. *N Engl J Med* 2009; **360**: 129-139 [PMID: 19092145 DOI: 10.1056/NEJMoa0808431]
  - 68 **Patel A**, MacMahon S, Chalmers J, Neal B, Billot L, Woodward M, Marre M, Cooper M, Glasziou P, Grobbee D, Hamet P, Harrap S, Heller S, Liu L, Mancia G, Mogensen CE, Pan C, Poulter N, Rodgers A, Williams B, Bompont S, de Galan BE, Joshi R, Travert F. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. *N Engl J Med* 2008; **358**: 2560-2572 [PMID: 18539916 DOI: 10.1056/NEJMoa0802987]
  - 69 **Wu JH**, Haan MN, Liang J, Ghosh D, Gonzalez HM, Herman WH. Impact of antidiabetic medications on physical and cognitive functioning of older Mexican Americans with diabetes mellitus: a population-based cohort study. *Ann Epidemiol* 2003; **13**: 369-376 [PMID: 12821276 DOI: 10.1016/S1047-2797(02)00464-7]
  - 70 **Launer LJ**, Miller ME, Williamson JD, Lazar RM, Gerstein HC, Murray AM, Sullivan M, Horowitz KR, Ding J, Marcovina S, Lovato LC, Lovato J, Margolis KL, O'Connor P, Lipkin EW, Hirsch J, Coker L, Maldjian J, Sunshine JL, Truwit C, Davatzikos C, Bryan RN. Effects of intensive glucose lowering on brain structure



- and function in people with type 2 diabetes (ACCORD MIND): a randomised open-label substudy. *Lancet Neurol* 2011; **10**: 969-977 [PMID: 21958949 DOI: 10.1016/S1474-4422(11)70188-0]
- 71 **Kawamura T**, Umemura T, Hotta N. Cognitive impairment in diabetic patients: Can diabetic control prevent cognitive decline? *J Diabetes Investig* 2012; **3**: 413-423 [PMID: 24843599 DOI: 10.1111/j.2040-1124.2012.00234.x]
  - 72 **Mathur S**, Zammitt NN, Frier BM. Optimal glycaemic control in elderly people with type 2 diabetes: what does the evidence say? *Drug Saf* 2015; **38**: 17-32 [PMID: 25481812 DOI: 10.1007/s40264-014-0247-7]
  - 73 **Rizzo MR**, Marfella R, Barbieri M, Boccardi V, Vestini F, Lettieri B, Canonico S, Paolisso G. Relationships between daily acute glucose fluctuations and cognitive performance among aged type 2 diabetic patients. *Diabetes Care* 2010; **33**: 2169-2174 [PMID: 20573753 DOI: 10.2337/dc10-0389]
  - 74 **Abbatecola AM**, Rizzo MR, Barbieri M, Grella R, Arciello A, Laieta MT, Acampora R, Passariello N, Cacciapuoti F, Paolisso G. Postprandial plasma glucose excursions and cognitive functioning in aged type 2 diabetics. *Neurology* 2006; **67**: 235-240 [PMID: 16864814 DOI: 10.1212/01.wnl.0000224760.22802.e8]
  - 75 **Dandona P**. Endothelium, inflammation, and diabetes. *Curr Diab Rep* 2002; **2**: 311-315 [PMID: 12643190 DOI: 10.1007/s11892-002-0019-0]
  - 76 **Matsuzaki T**, Sasaki K, Tanizaki Y, Hata J, Fujimi K, Matsui Y, Sekita A, Suzuki SO, Kanba S, Kiyohara Y, Iwaki T. Insulin resistance is associated with the pathology of Alzheimer disease: the Hisayama study. *Neurology* 2010; **75**: 764-770 [PMID: 20739649 DOI: 10.1212/WNL.0b013e3181ee25f]
  - 77 **Craft S**, Asthana S, Cook DG, Baker LD, Cherrier M, Purganan K, Wait C, Petrova A, Latendresse S, Watson GS, Newcomer JW, Schellenberg GD, Krohn AJ. Insulin dose-response effects on memory and plasma amyloid precursor protein in Alzheimer's disease: interactions with apolipoprotein E genotype. *Psychoneuroendocrinology* 2003; **28**: 809-822 [PMID: 12812866 DOI: 10.1016/S0306-4530(02)00087-2]
  - 78 **Imfeld P**, Bodmer M, Jick SS, Meier CR. Metformin, other antidiabetic drugs, and risk of Alzheimer's disease: a population-based case-control study. *J Am Geriatr Soc* 2012; **60**: 916-921 [PMID: 22458300 DOI: 10.1111/j.1532-5415.2012.03916.x]
  - 79 **Ruis C**, Biessels GJ, Gorter KJ, van den Donk M, Kappelle LJ, Rutten GE. Cognition in the early stage of type 2 diabetes. *Diabetes Care* 2009; **32**: 1261-1265 [PMID: 19366968 DOI: 10.2337/dc08-2143]
  - 80 **Gaede P**, Lund-Andersen H, Parving HH, Pedersen O. Effect of a multifactorial intervention on mortality in type 2 diabetes. *N Engl J Med* 2008; **358**: 580-591 [PMID: 18256393 DOI: 10.1056/NEJMoa0706245]

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Baishideng Publishing Group Inc  
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Telephone: +1-925-2238242  
Fax: +1-925-2238243  
E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
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## Update on pre-diabetes: Focus on diagnostic criteria and cardiovascular risk

Antonino Di Pino, Francesca Urbano, Salvatore Piro, Francesco Purrello, Agata Maria Rabuazzo

Antonino Di Pino, Francesca Urbano, Salvatore Piro, Francesco Purrello, Agata Maria Rabuazzo, Department of Clinical and Experimental Medicine, University of Catania, 95122 Catania, Italy

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**Correspondence to:** Francesco Purrello, MD, Department of Clinical and Experimental Medicine, University of Catania, Via Palermo, 95122 Catania, Italy. [fpurrell@unict.it](mailto:fpurrell@unict.it)  
Telephone: +39-09-57598401  
Fax: +39-09-57598421

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### Abstract

Pre-diabetes, which is typically defined as blood glucose concentrations higher than normal but lower than the

diabetes threshold, is a high-risk state for diabetes and cardiovascular disease development. As such, it represents three groups of individuals: Those with impaired fasting glucose (IFG), those with impaired glucose tolerance (IGT) and those with a glycated haemoglobin (HbA<sub>1c</sub>) between 39-46 mmol/mol. Several clinical trials have shown the important role of IFG, IGT and HbA<sub>1c</sub>-pre-diabetes as predictive tools for the risk of developing type 2 diabetes. Moreover, with regard to cardiovascular disease, pre-diabetes is associated with more advanced vascular damage compared with normoglycaemia, independently of confounding factors. In view of these observations, diagnosis of pre-diabetes is mandatory to prevent or delay the development of the disease and its complications; however, a number of previous studies reported that the concordance between pre-diabetes diagnoses made by IFG, IGT or HbA<sub>1c</sub> is scarce and there are conflicting data as to which of these methods best predicts cardiovascular disease. This review highlights recent studies and current controversies in the field. In consideration of the expected increased use of HbA<sub>1c</sub> as a screening tool to identify individuals with alteration of glycaemic homeostasis, we focused on the evidence regarding the ability of HbA<sub>1c</sub> as a diagnostic tool for pre-diabetes and as a useful marker in identifying patients who have an increased risk for cardiovascular disease. Finally, we reviewed the current evidence regarding non-traditional glycaemic biomarkers and their use as alternatives to or additions to traditional ones.

**Key words:** Glycated haemoglobin; Cardiovascular risk; Diagnostic criteria; Non-traditional glycaemic markers; Pre-diabetes

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**Core tip:** Pre-diabetes is a high-risk state for diabetes and cardiovascular disease. There are three diagnostic criteria for pre-diabetes: Impaired fasting glucose (IFG),



impaired glucose tolerance (IGT) and glycated haemoglobin (HbA<sub>1c</sub>) between 39-46 mmol/mol. The concordance between a pre-diabetes diagnosis made by IFG, IGT or HbA<sub>1c</sub> is scarce and there are conflicting data as to which of these methods best predicts cardiovascular disease. This review focuses on the evidence regarding the ability of HbA<sub>1c</sub> for pre-diabetes diagnosis and as a marker for cardiovascular risk. Finally, the evidence regarding non-traditional glycaemic biomarkers as alternatives to the traditional ones is reviewed.

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## INTRODUCTION

Pre-diabetes is a general term that refers to an intermediate stage between normal glucose homeostasis and overt type 2 diabetes mellitus. As such, it includes three groups of individuals: Those with impaired fasting glucose (IFG), those with impaired glucose tolerance (IGT) and those with a glycated hemoglobin (HbA<sub>1c</sub>) between 39-46 mmol/mol (Table 1). As underlined by the American Diabetes Association (ADA), a number of previous studies reported that the concordance between pre-diabetes diagnoses made by IFG, IGT or HbA<sub>1c</sub> is scarce<sup>[1]</sup>; according with this consideration, in a study conducted on a large population of Caucasian adults the agreement between the three diagnostic criteria was only 10.4% (Figure 1)<sup>[2]</sup>.

The discordance in the identification of individuals with pre-diabetes using three different diagnostic tests is not entirely unexpected given that fasting plasma glucose, 2 h post oral glucose tolerance test (OGTT), and HbA<sub>1c</sub> probably reflect different aspects of glucose metabolism, and a diagnosis of pre-diabetes based on IFG, IGT, or HbA<sub>1c</sub> may represent aetiological factors leading to the development of the different prediabetic states<sup>[2]</sup>. Indeed, subjects with isolated IFG seem to have a reduced hepatic insulin sensitivity, impaired first-phase insulin secretion, and normal/near-normal muscle insulin sensitivity, while subjects with IGT should be characterized by nearly normal hepatic insulin sensitivity and marked reduced peripheral insulin sensitivity combined with defective late insulin secretion<sup>[3,4]</sup>. In contrast to IFG and IGT, HbA<sub>1c</sub> is a marker representing blood glucose concentrations over the preceding 2-3 mo and it is affected by both basal and postprandial hyperglycaemia. To date, it is still not clear if these aspects that are strictly bound to the physiopathology of pre-diabetes may have a clinical relevance in view of a possible therapeutic intervention.

Cardiovascular disease (CVD) is the leading cause of death among individuals with type 2 diabetes, accounting

for 40% to 50% of all deaths<sup>[5]</sup>. Although type 2 diabetes is frequently associated with other cardiovascular risk factors, such as dyslipidemia and hypertension, it is believed that chronic hyperglycaemia *per se* is an independent risk for macrovascular complications. Currently, it is well established that macrovascular disease starts before the development of diabetes, and the slight increase in plasma glucose levels that characterize pre-diabetes have been shown to be an independent predictor for CVD. Much clinical research has focused on lifestyle or pharmacological intervention to prevent diabetes in these high risk subjects<sup>[6]</sup>; however, few studies have been conducted with specific focus on CVD prevention in this population. Since many clinical trials have failed to demonstrate a reduction in cardiovascular risk from glucose-lowering interventions in patients with overt type 2 diabetes<sup>[7,8]</sup>, it is noteworthy that several studies have reported benefits in improving cardiovascular risk factors, as well as absolute CVD event rates, in people with pre-diabetes treated with glucose lowering drugs<sup>[9-11]</sup>.

Since the utility of a test for pre-diabetes diagnosis is also defined by its capacity to identify the macrovascular complication risk, an important question is whether subjects with pre-diabetes according to IFG, IGT, or HbA<sub>1c</sub> have an equivalent cardiovascular risk. To date, cardiovascular risk studies comparing IFG, IGT, and HbA<sub>1c</sub>-pre-diabetic patients are sparse and the results are still controversial<sup>[12-14]</sup>.

This review highlights recent studies and current controversies in the field. In consideration of the increased use of HbA<sub>1c</sub> as a marker to detect patients with alterations of glycaemic homeostasis, we thought that it could be interesting, and relevant from the clinical point of view, to evaluate the evidence regarding the ability of HbA<sub>1c</sub> to identify patients who have increased cardiovascular risk. With this specific aim we focused our attention on HbA<sub>1c</sub> as a diagnostic tool for pre-diabetes. Finally, we reviewed the current evidence regarding non-traditional glycaemic biomarkers and their use as alternatives to or additions to the traditional ones.

## COMPARISON OF IFG, IGT AND HBA<sub>1c</sub>, CRITERIA IN PREDICTING TYPE 2 DIABETES

Subjects with pre-diabetes have shown a high conversion rate to overt diabetes and much clinical research has focused on lifestyle or pharmacological intervention to prevent diabetes in these high risk subjects<sup>[6]</sup>. Subjects with an isolated alteration of glucose homeostasis (IFG, IGT or HbA<sub>1c</sub> 39-46 mmol/mol) have an incidence of diabetes of 6% per year, a value that is significantly higher compared with subjects with normoglycemia (0.5% per year)<sup>[15]</sup>. Progression to overt type 2 diabetes is 30%-40% in the next 3-8 years, with an increase of 10% when two alterations of glucose homeostasis are present<sup>[6]</sup>.

According with these considerations, diagnostic and

**Table 1** Diagnostic criteria for categories at increased risk of diabetes

Category	Marker	Diagnostic range
IFG	Fasting plasma	$\geq 5.6$ mmol/L (100 mg/dL)
	glycemia	$< 6.9$ mmol/L (126 mg/dL)
IGT	2-h post-load	$\geq 7.8$ mmol/L (140 mg/dL)
	glycemia	$< 11$ mmol/L (200 mg/dL)
HbA <sub>1c</sub> -prediabetes	HbA <sub>1c</sub>	$\geq 39$ mmol/mol (5.7%)
		$< 47$ mmol/mol (6.5%)

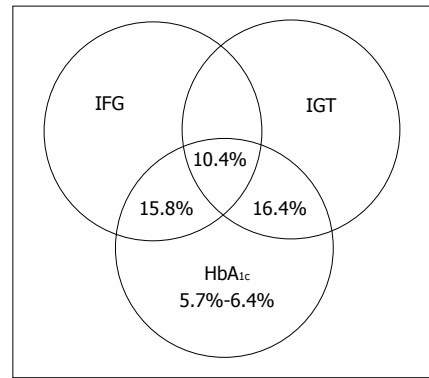
IFG: Impaired fasting glucose; IGT: Impaired glucose tolerance; HbA<sub>1c</sub>: Glycated haemoglobin.

screening criteria for pre-diabetes have a relevant clinical impact; indeed, it is important to identify individuals at high risk for type 2 diabetes to prevent or delay the development of the disease and its complications.

In 2011, the ADA revised the criteria for the diagnosis of type 2 diabetes and the categories at increased risk for diabetes and the use of HbA<sub>1c</sub> measurement was recommended as another diagnostic test option already including IFG and IGT<sup>[1]</sup>. Specifically for the categories of increased risk for type 2 diabetes, the new ADA recommendations state that an HbA<sub>1c</sub> from 39–46 mmol/mol identifies individuals at high risk for diabetes to whom the term pre-diabetes may be applied.

Indeed, both IFG and IGT present some limitations: They require fasting status and are affected by acute perturbations. Furthermore, the OGTT presents some practical difficulties: It is costly, it needs time, and has lower reproducibility compared with the fasting plasma glucose measurement (FPG)<sup>[16]</sup>. HbA<sub>1c</sub> is a “picture” of the average blood glucose level over the period of 2–3 mo<sup>[17]</sup>. HbA<sub>1c</sub> has higher reproducibility than FPG; indeed, within subject coefficients of variation are 1.7% for HbA<sub>1c</sub>, and 5.7% for FPG<sup>[17,18]</sup>. Furthermore, HbA<sub>1c</sub> does not need fasting status and could better integrate chronic hyperglycaemia than FPG (Table 2). The predictive value of HbA<sub>1c</sub> for type 2 diabetes has been reported in several studies. Morris *et al.*<sup>[19]</sup> has shown in a metanalysis conducted on 70 studies that the progression rate to type 2 diabetes of patients with HbA<sub>1c</sub> pre-diabetes was similar to that for ADA-defined IFG and IFG plus IGT. Moreover, the value of HbA<sub>1c</sub> in predicting type 2 diabetes has been reported four prospective studies<sup>[20–23]</sup>; of these, one assessed the use of two glycemic parameters (in particular IFG and HbA<sub>1c</sub>) for predicting the incidence of type 2 diabetes; the authors supported the combined measurement of FPG and HbA<sub>1c</sub> for predicting diabetes incidence in a 4 year follow-up using receiver operating characteristic curve (ROC) analysis. When the whole population was analysed, the ROC curve of the model including both FPG and HbA<sub>1c</sub> was greater those including FPG alone or HbA<sub>1c</sub> alone. Furthermore, the authors reported a weak correlation between HbA<sub>1c</sub> and FPG at baseline suggesting that HbA<sub>1c</sub> is not a surrogate marker of FPG<sup>[23]</sup>.

It is necessary to remember that HbA<sub>1c</sub> between



**Figure 1** Agreement between glycated haemoglobin pre-diabetes, impaired fasting glucose and impaired glucose tolerance<sup>[2]</sup>. IFG: Impaired fasting glucose; IGT: Impaired glucose tolerance; HbA<sub>1c</sub>: Glycated haemoglobin.

39–46 mmol/mol seems to have a lower sensitivity in identify population with pre-diabetes compared with IFG and IGT<sup>[24,25]</sup>. Conversely, the use of HbA<sub>1c</sub> may also lead to the reclassification of subjects without IFG or IGT as having pre-diabetes<sup>[26]</sup>. On the other hand, according to the ADA statement, the lower sensitivity of HbA<sub>1c</sub> for diagnosing pre-diabetes may be offset by its ability to facilitate establishing a diagnosis<sup>[27]</sup>. Contrary to these considerations, Rosella *et al.*<sup>[28]</sup> recently reported that the prevalence of undiagnosed pre-diabetes in a representative sample of Canadians was significantly higher using HbA<sub>1c</sub> measures as screening tool compared with plasma glucose diagnostic criteria. The authors hypothesized that this “reverse association” may be due to a number of factors, such as ethnic differences and the increased prevalence of pre-diabetes from 11.6% in 2003 to 35.3% in 2011<sup>[29]</sup>. Accordingly, in a study conducted in the Mexican population, Kumar *et al.*<sup>[30]</sup> found a higher prevalence of adults with HbA<sub>1c</sub> pre-diabetes compared with previous studies conducted in the same population<sup>[31]</sup>. We reported similar findings in a recent study conducted on 380 subjects attending our out-patients clinic for diabetes and cardiovascular risk evaluation; although we did not perform an opportunistic procedure during recruitment, the group with high HbA<sub>1c</sub> and normal fasting glucose and normal glucose tolerance (NFG/NGT) represented, in this study, approximately 30% of the entire population and is, therefore, not a rare subset<sup>[32]</sup>. These observations may not be surprising; in fact, although subjects with NFG and NGT have a lower risk of developing diabetes than patients with either IFG or IGT, in several studies a significant percentage (30%–40%) of all individuals who developed type 2 diabetes had NFG and NGT at baseline<sup>[33,34]</sup>. This indicates that subjects with NFG and NGT experience a lower risk of developing diabetes compared with IFG and IGT in absolute terms; however, among these subjects there is also a subgroup at increased risk of developing diabetes and, consequently, cardiovascular diseases. From these considerations stems the need to add HbA<sub>1c</sub>, as a diagnostic tool to identify a new category of high-risk individuals<sup>[35]</sup>. Further epidemiological data are needed to characterize the real percentage of this group

**Table 2** Main points supporting/not supporting the use of glycated haemoglobin as diagnostic tool for diagnosis of pre-diabetes

Supporting	Not supporting
HbA <sub>1c</sub> may better integrate chronic hyperglycaemia than fasting and 2-h post-load glycaemia	HbA <sub>1c</sub> seems to have a lower sensitivity in pre-diabetes diagnosis
HbA <sub>1c</sub> predicts microvascular complications (retinopathy and nephropathy) similarly to fasting and 2-h post-load glycaemia	Standardization of HbA <sub>1c</sub> assay needs to be improved
HbA <sub>1c</sub> has a higher predictive value than fasting plasma glucose in predicting cardiovascular disease	Common, and not always known, clinical conditions (haemoglobinopathies, malaria, anaemia, blood loss) may significantly interfere with HbA <sub>1c</sub> assay
HbA <sub>1c</sub> has a greater pre-analytical stability than blood glucose	Ethnic differences in HbA <sub>1c</sub> assay are not well characterized
HbA <sub>1c</sub> assay does not need fasting status	The low biological variability of HbA <sub>1c</sub> provides little information on pathophysiological processes involved in pre-diabetes
HbA <sub>1c</sub> is not affected by acute perturbations (exercise, stress, diet)	Glucose assessment is cheaper than HbA <sub>1c</sub> assay
HbA <sub>1c</sub> biological variability is lower than fasting and 2-h post-load glycaemia	
HbA <sub>1c</sub> may be an attractive option in settings in which OGTT is not used and rarely repeated	

HbA<sub>1c</sub>: Glycated haemoglobin; OGTT: Oral glucose tolerance test.

in the overall pre-diabetic population.

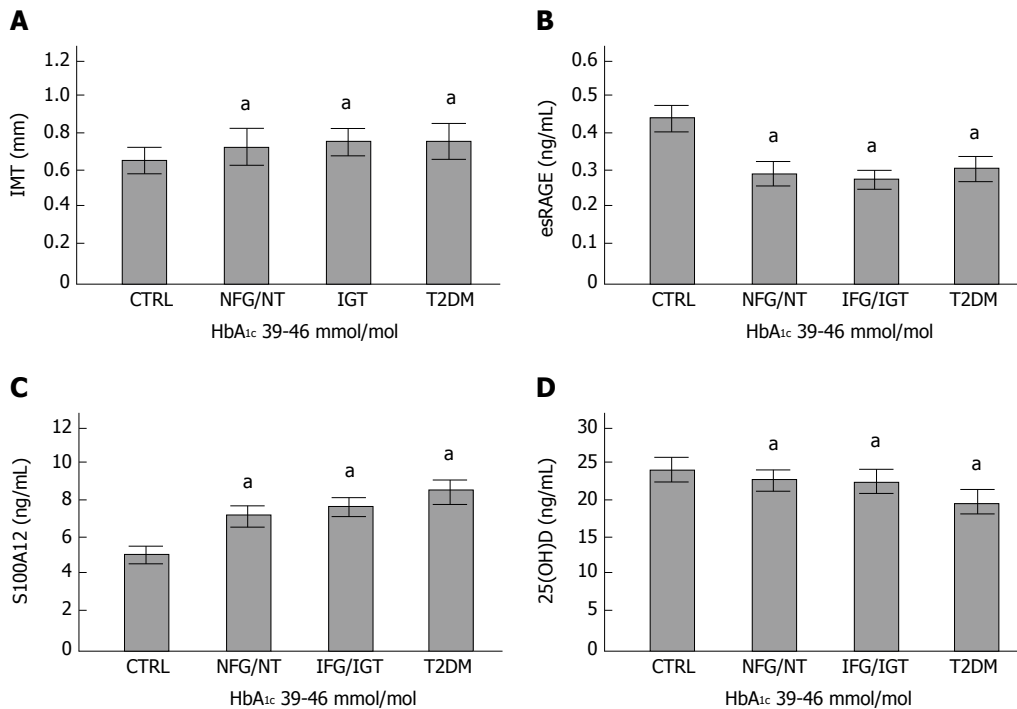
To date, it is unclear why the prevalence of pre-diabetes diagnosed by OGTT and HbA<sub>1c</sub> criteria is substantially discordant. The concentration of HbA<sub>1c</sub> depends on glucose concentrations and on factors affecting the glycation rate such as systemic oxidative stress. Previous studies reported that some characteristics, such as obesity, are associated with increased oxidative stress<sup>[36]</sup>, thus, HbA<sub>1c</sub> may not reflect the real concentration of glucose and be disproportionately high in obese subjects. Several studies investigated the effects of phenotypic characteristics such as obesity on the agreement between OGTT and HbA<sub>1c</sub>. Li *et al.*<sup>[37]</sup> in a recent study conducted on a large cohort of Chinese subjects without a previous diagnosis of diabetes reported a poor agreement between HbA<sub>1c</sub> criteria and OGTT in patients independently from body mass index. Moreover, different optimal HbA<sub>1c</sub> cut-off points for pre-diabetes were reported: 38 mmol/mol for normal weight, 39 mmol/mol for overweight, and 42 mmol/mol for obese subjects.

Also other studies recommend a different cut-off point of HbA<sub>1c</sub> for diagnosis of pre-diabetes. In particular, longitudinal epidemiological studies have reported that demographic and ethnic factors may contribute to complications in using HbA<sub>1c</sub> for the diagnosis of diabetes, and the optimal diagnostic HbA<sub>1c</sub> value is debated and varies because of genetic and biological differences. Yan *et al.*<sup>[38]</sup> identified optimal HbA<sub>1c</sub> cut-off points for pre-diabetes in two diverse population-based cohorts with different ages. The optimal HbA<sub>1c</sub> cut-off point for pre-diabetes diagnosis was 38 mmol/mol in the young and middle-aged population, whereas, the optimal cut-off for diagnosing pre-diabetes increased to 39 mmol/mol, in the elderly population. Furthermore, many studies have shown that racial disparities affect the performance of HbA<sub>1c</sub> for diagnosing pre-diabetes<sup>[39]</sup>. In summary, it is possible that diagnostic tests for glycemic homeostasis should be used and interpreted considering the individual phenotypic characteristics of the patients; further studies are needed to investigate the clinical usefulness of personalized cutoff values.

## COMPARISON OF IFG, IGT AND HbA<sub>1c</sub> CRITERIA IN PREDICTING CARDIOVASCULAR RISK

The utility of a test for pre-diabetes diagnosis is also defined by its capacity to identify the risk of micro- and macrovascular complications and from this point of view, the high reproducibility and simplicity may make HbA<sub>1c</sub> dosage an attractive option. Previous observational studies documented that determination of HbA<sub>1c</sub>, fasting glucose and OGTT significantly predicted the development of retinopathy and nephropathy but no variables had a significant advantage for detecting the incidence or prevalence of either complication<sup>[40,41]</sup>. However, fasting glycaemia has a low predictive value in terms of cardiovascular disease, while 2-h post-load glycaemia and HbA<sub>1c</sub> have a higher predictive value for this chronic complication of diabetes<sup>[42]</sup>.

In a recent work, we showed that arterial stiffness and carotid intima-media thickness were altered in subjects with higher HbA<sub>1c</sub> levels and similar as that observed in subjects with new onset type 2 diabetes<sup>[43]</sup>. Furthermore, when we analyzed our population including only subjects with NFG/NGT we found that the NFG/NGT subjects with HbA<sub>1c</sub> 39-46 mmol/mol showed an alteration of subclinical markers of cardiovascular risk compared with NFG/NGT with lower HbA<sub>1c</sub> and no significant differences were found compared with IGT and type 2 diabetic patients (Figure 2). According to these data, a reproducible and simple marker such as HbA<sub>1c</sub> seems to identify subjects at high cardiovascular risk that would be considered normal according to fasting glycaemia and glucose tolerance. Other studies have shown similar data reporting a positive association between the pre-diabetic stage, echogenic plaque and progression of coronary artery calcification<sup>[44,45]</sup>. A recent study has analysed the routine use of HbA<sub>1c</sub> for diagnosis of pre-diabetes in patients with ST-segment elevation myocardial infarction. The study showed a similar in-hospital and long-term mortality in these patients with pre-diabetes as those with known diabetes. The authors discussed that the



**Figure 2** Intima media thickness, endogenous receptor for advanced glycation end-products, S100A12 and 5-hydroxyvitamin D according to glucose tolerance and glycated haemoglobin levels. A: IMT, <sup>a</sup> $P < 0.05$  vs CTRL; B: esRAGE, <sup>a</sup> $P < 0.05$  vs CTRL; C: S100A12, <sup>a</sup> $P < 0.05$  vs CTRL; D: 25(OH)D, <sup>a</sup> $P < 0.05$  vs CTRL. IMT: Intima-media thickness; esRAGE: Endogenous receptor for advanced glycation end-products; 25(OH)D: 25-hydroxyvitamin D; NFG: Normal fasting glucose; NGT: Normal glucose tolerance; IFG: Impaired fasting glucose; IGT: Impaired glucose tolerance; T2DM: Type 2 diabetes mellitus; HbA<sub>1c</sub>: Glycated haemoglobin.

difficulty in performance and the presence of stress hyperglycaemia in an acutely ill patient with myocardial infarction make OGTT a rarely used diagnostic test in this setting. The use of a simple, one-time HbA<sub>1c</sub> test allowed them to identify a substantial proportion of patients with previously undiagnosed diabetes or pre-diabetes who could be targeted for risk factor modification with lifestyle interventions and tailored medical therapy<sup>[46]</sup>.

The links between alteration of glucose homeostasis and vascular damage in this population is still unclear, however, several studies have emphasized that the interaction of advanced glycation end products (AGE) with their cell-surface receptor (RAGE) is implicated in triggering inflammatory processes strictly connected with cardiovascular disease<sup>[47]</sup>. A RAGE soluble form termed endogenous secretory RAGE (esRAGE) may contribute to the removal of circulating ligands, thus competing with cell-surface RAGE for ligand binding<sup>[48]</sup>. Low levels of esRAGE have been associated with cardiovascular disease and, in a recent study, we found that subjects with pre-diabetes showed low esRAGE plasma levels suggesting a decreased scavenger capacity of these subjects (Figure 2). Further analysis conducted on mononuclear cells isolated from peripheral blood samples of these patients revealed a decreased esRAGE mRNA expression<sup>[32]</sup>. The regulatory mechanism for alternative splicing to generate esRAGE remains unclear, and environmental or genetic factors may be involved. Further examinations of the molecular mechanism underlying esRAGE regulation will provide potential targets for the prevention and/or treatment of cardiovascular disease.

Our research team has further investigated the characterization of the population with HbA<sub>1c</sub> pre-diabetes (39-46 mmol/mol) also investigating other markers closely associated with metabolic abnormalities and cardiovascular risk; in a previous study we highlighted a reduced insulin response in combination with impaired suppression of glucagon secretion in subjects with pre-diabetes according to HbA<sub>1c</sub> undergoing isoglycaemic intravenous glucose infusion<sup>[49]</sup>. Other data published in 2014 indicated that the presence of pre-diabetes according to HbA<sub>1c</sub> is associated with hepatic steatosis and with an alteration in the lipid profile known to be predisposing to cardiovascular and liver diseases<sup>[50]</sup>. Moreover, we showed that the levels of 25 hydroxyvitamin D are reduced and associated with vascular damage in subjects with pre-diabetes by HbA<sub>1c</sub> with NFG/NGT (Figure 2)<sup>[51]</sup>. Based on these data, we suggest that among subjects with NFG and NGT, HbA<sub>1c</sub> may identify subjects with different cardiovascular and glycometabolic risks.

These considerations are, furthermore, supported by previous studies. Indeed, it is important to remember that many authors have documented a significant increase in the incidence of cardiovascular events with HbA<sub>1c</sub> values substantially lower than those used for diagnosis of diabetes<sup>[12]</sup>. A recent meta-analysis of six prospective cohort studies in subjects without diabetes mellitus showed a linear association of HbA<sub>1c</sub> levels with primary cardiovascular events. The observed effect estimates for increased HbA<sub>1c</sub> levels and was strongly attenuated by adjustment for cardiovascular risk factors but remained statistically significant for primary car-



diovascular events, cardiovascular mortality and all-cause mortality<sup>[52]</sup>.

The majority of randomized controlled trials in non-diabetic subjects with increased HbA<sub>1c</sub> failed to observe significant effects when aiming to reduce the cardiovascular risk and mortality of these individuals. In the recent IRIS trial, which involved patients without diabetes but with a recent history of ischemic stroke or transitory ischemic attack and who had insulin resistance, the rate of the primary outcome (fatal or non-fatal stroke or fatal or non-fatal myocardial infarction) was lower in the pioglitazone group compared with placebo<sup>[11]</sup>. These results, although in contrast, at least in part, with other trials conducted on patients with type 2 diabetes (BARI-2D and Pro-active), are of great interest suggesting a favourable effect of pioglitazone on the progression of subclinical atherosclerosis<sup>[53,54]</sup>. The mechanism that was responsible for the lower rates of stroke and myocardial infarction in the pioglitazone group remains unclear. A recent meta-analysis of prospective, randomized clinical trials has shown a non-significant trend towards reduced risk of fatal and non-fatal myocardial infarction, and fatal and non-fatal stroke were only reduced to borderline. However, the short average follow-up time of 3.75 years was a limitation of previous trials and further RCTs, with a larger sample size and longer follow-up, are required to explore the efficacy of non-drug and drug based approaches to reduce the cardiovascular risk of non-diabetic subjects with increased HbA<sub>1c</sub><sup>[55]</sup>.

Other studies have reported similar findings suggesting the role of HbA<sub>1c</sub> as an early marker of cardiovascular risk; however, it is pertinent to recognize that the determinants of cardiovascular risk in subjects with metabolic alterations are complex and multiple, and individual's cardiovascular risk can't be identified by a single laboratory test<sup>[56]</sup>.

## BEYOND TRADITIONAL DIAGNOSTIC CRITERIA: THE ROLE OF NON-TRADITIONAL GLYCAEMIC MARKERS IN PREDICTING DIABETES AND CARDIOVASCULAR RISK

As previously explained, the traditional markers of glucose homeostasis are not definitive, and their use in clinical practice may be biased by a number of clinical and analytical factors. For these reasons, there is growing interest in new serum biomarkers of hyperglycaemia to be used as alternatives or in conjunction with traditional measures. In this review, we will provide a brief overview of the properties and of the existing literature linking these emerging biomarkers with micro- and macro-vascular complications.

### *One-hour post-load plasma glucose*

Recently, an increasing body of evidence has focused on subjects with a plasma glucose concentration of at least

8.6 mmol/L at 1-h during OGTT. In 2008, Abdul-Ghani *et al.*<sup>[57]</sup> demonstrated for the first time that the 1-h post-load plasma glucose concentration may be a clinical indicator that can be used to identify subjects with high risk for type 2 diabetes. These observations were confirmed in other recent studies showing that the incidence rate to type 2 diabetes over a period of 5 years in subjects with NGT and 1-h post-load glycaemia > 8.6 mmol/L was 16.7%<sup>[58]</sup>. Furthermore, a 1-h post-load glycaemia value > 8.6 mmol/L was strongly associated with different predictors for future cardiovascular events<sup>[59,60]</sup>. In conclusion, it seems that this glucose value may identify subjects with an intermediate cardiometabolic risk profile between NGT and IGT<sup>[57,61]</sup>. This has been observed and confirmed in populations of different ethnicities such as Mexican-American, Scandinavian Caucasian, and Asian Indian<sup>[59,61,62]</sup>. Why 1-h post-load glucose is a good indicator of cardiometabolic risk is still an open question; to date it is known that chronic hyperglycaemia promotes the formation of advanced glycation end products and reactive oxygen species.

One hour post-load glycaemia provides physiopathological information since it is dependent on insulin sensitivity in skeletal muscles and beta-cell function<sup>[63]</sup>.

These data might underline the importance of obtaining intermediate plasma glucose levels during oral glucose tolerance test<sup>[59,64]</sup>. However, from the clinical point of view, 1-h post-load glycaemia requires, in any case, an OGTT, and, to date, strict lifestyle modification is the only therapy recommended from guidelines for subjects with pre-diabetes, independently from their physiopathologic profile. Furthermore, a study conducted on subjects with HbA<sub>1c</sub> pre-diabetes reported that most patients with HbA<sub>1c</sub> in the 39-46 mmol/mol range have a 1-h glucose  $\geq$  8.6 mmol/L; these data lead to the consideration that HbA<sub>1c</sub> may be the most practical tool to identify subjects with impaired glucose homeostasis<sup>[43]</sup>.

### *Fructosamine and glycated albumin*

Fructosamine and glycated albumin are both ketoamines formed from the binding of fructose to total serum protein, mostly albumin, through glycosylation. The fructosamine assay is cheaper and easier to perform than the HbA<sub>1c</sub> assay and it measures total glycated serum protein, whereas glycated albumin is reported as the proportion of total albumin<sup>[65]</sup>. Fructosamine and glycated albumin are short-term markers of glucose homeostasis; indeed, they provide information on blood glucose levels over the previous 2-3 wk<sup>[66]</sup>. This depends on the rapid turnover of glycated proteins, that in contrast to HbA<sub>1c</sub>, is independent from the turnover of red blood cells or hemoglobin characteristics. Similar to HbA<sub>1c</sub>, blood for fructosamine dosage can be obtained in any moment of the day, without regard to recent food intake. Both fructosamine and glycated albumin are associated with future risk of diabetes, independently from fasting glucose and HbA<sub>1c</sub><sup>[67,68]</sup>. Another recent study explored the ability of HbA<sub>1c</sub>, fructosamine and glycated albumin to detect pre-

diabetes and whether there would be added diagnostic value in combining HbA<sub>1c</sub> with fructosamine or glycated albumin. The study, conducted on United States Africans, showed that HbA<sub>1c</sub>, fructosamine and glycated albumin detected almost 50% of Africans with pre-diabetes; however, combining HbA<sub>1c</sub> with glycated albumin (but not with fructosamine) made it possible to identify nearly 80% of Africans with pre-diabetes, as reported in previous studies<sup>[69]</sup>. Furthermore, the authors reported that pre-diabetic patients identified by glycated protein were younger and with a lower BMI, as previously reported. It is still not clear why glycated plasma proteins are inversely related to body size, however, this observation could be of clinical relevance and it may support the use of glycated albumin to enhance the detection of pre-diabetes in specific populations, such as the non-obese.

Evidence derived from prospective studies regarding the link between non-traditional markers and micro and macrovascular complications are limited. Data from the Atherosclerosis Risk in Communities (ARIC) Study have shown that glycated albumin predicted chronic kidney disease over two decades of follow-up with a similar magnitude to those observed for HbA<sub>1c</sub><sup>[69]</sup>. Other evidence has come from cross-sectional studies. A recent analysis from the ARIC Study has shown an association between glycated albumin and retinopathy, with a pattern of association very similar to that observed for HbA<sub>1c</sub><sup>[69]</sup>. Furthermore, in other studies conducted on adults without diagnosed diabetes, glycated albumin was associated with subclinical atherosclerosis, kidney and cardiovascular disease<sup>[70]</sup>.

A potential limitation to the clinical use of these markers may be that, to date, there is no established clinical cut-off points and the assays are not standardized across instruments. Particular caution should be used in pathological conditions that can impact albumin metabolism including anaemia, malnutrition, nephrotic syndrome and liver cirrhosis.

To date, fructosamine and glycated albumin are not incorporated in clinical guidelines, however, they may be useful complements to HbA<sub>1c</sub> in clinical practice, mainly when HbA<sub>1c</sub> testing is inaccessible or when the result might not be reliable.

### 1,5-anhydroglucitol

1,5-anhydroglucitol (1,5-AG) is a monosaccharide primarily derived from dietary sources and is a non-traditional biomarker of hyperglycaemia. During euglycaemia, serum 1,5-AG is typically maintained at a constant concentration (12-40 µg/mL). It is freely filtered from the glomeruli and a small amount, dependent on dietary intake, is excreted with the urine. The remaining amount is reabsorbed in the renal tubule. In conditions of hyperglycaemia (> 8.9-10 mmol/L) glucose blocks renal tubular reabsorption of 1,5-AG resulting in a drop in 1,5-AG serum levels; therefore, an inverse association exists between hyperglycaemia and 1,5-AG. Clinically, 1,5-AG may be used as a marker of short-term glycaemic variability, reflecting

hyperglycaemic episodes over 1-2 wk. 1,5-AG is a non-fasting test and it may include information about glycaemic excursion that is not included in HbA<sub>1c</sub> dosage.

Previous studies found a significant association between 1,5-AG and the subsequent development of diabetes with a magnitude that was significant but weaker compared with fructosamine and glycated albumin<sup>[68]</sup>. However, consistent with its pathophysiology, 1,5-AG was no longer associated with incident diabetes among people with a normal fasting glucose < 5.6 mmol/L or HbA<sub>1c</sub> < 39 mmol/mol, suggesting a limited usefulness for 1,5-AG in the setting of normal glucose and HbA<sub>1c</sub> levels. According to this data 1,5-AG seems to be a biomarker suitable for detecting glycaemic variations in patients with HbA<sub>1c</sub> between 53-64 mmol/mol (for example, to monitor a patient's response to changes in medication) rather than in subjects with pre-diabetes.

Few studies have assessed the relationship of 1,5-AG with micro and macrovascular complications. Cross-sectional studies have reported associations between 1,5-AG serum levels, subclinical atherosclerosis, prevalent retinopathy and coronary heart disease in subjects with and without diabetes<sup>[71,72]</sup>. A recent study observed a threshold effect, with little evidence of risk for cardiovascular events at the "non-diabetic" 1,5-AG concentration of 10-15 µg/mL. However, most of the study group were diabetic subjects, and in the categorical analysis the association with the clinical outcomes was largely confined to the subjects with diabetes<sup>[73]</sup>.

## CONCLUSION

The measurement of HbA<sub>1c</sub> appears to be a reliable diagnostic approach to identify patients at high risk for diabetes and cardiovascular disease; it seems to provide several advantages, especially in settings where OGTT is rarely used and never repeated as a confirmatory test, and eliminates a long series of biological and analytical limits. In most conditions HbA<sub>1c</sub> could become the reference method, provided that its assay is aligned with international standards. The budget/cost benefit of replacing glucose with HbA<sub>1c</sub> remains unclear and it is necessary to acquire additional information.

Finally, alternative biomarkers of glucose homeostasis may have a clinical use in identifying subjects at risk for diabetes and cardiovascular disease (mostly 1-h post-load glycaemia) and for short-term evaluation of glucose homeostasis in settings in which HbA<sub>1c</sub> may present some bias (fructosamine, glycated albumin and 1,5-AG). It is possible that one or more of these biomarkers may be of clinical usefulness, however, long-term prospective studies are needed to demonstrate whether their clinical use may be useful to improve outcomes and patient care.

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## REFERENCES

- American Diabetes Association.** Standards of medical care in diabetes--2014. *Diabetes Care* 2014; **37** Suppl 1: S14-S80 [PMID: 24357209 DOI: 10.2337/dc14-S014]
- Marini MA, Succurro E, Castaldo E, Cufone S, Arturi F, Sciacqua A, Lauro R, Hribal ML, Perticone F, Sesti G.** Cardiometabolic risk profiles and carotid atherosclerosis in individuals with pre-diabetes identified by fasting glucose, postchallenge glucose, and hemoglobin A1c criteria. *Diabetes Care* 2012; **35**: 1144-1149 [PMID: 22399698 DOI: 10.2337/dc11-2032]
- DeFronzo RA, Abdul-Ghani M.** Assessment and treatment of cardiovascular risk in prediabetes: impaired glucose tolerance and impaired fasting glucose. *Am J Cardiol* 2011; **108**: 3B-24B [PMID: 21802577 DOI: 10.1016/j.amjcard.2011.03.013]
- Abdul-Ghani MA, Tripathy D, DeFronzo RA.** Contributions of beta-cell dysfunction and insulin resistance to the pathogenesis of impaired glucose tolerance and impaired fasting glucose. *Diabetes Care* 2006; **29**: 1130-1139 [PMID: 16644654 DOI: 10.2337/diacare.2951130]
- de Marco R, Locatelli F, Zoppini G, Verlato G, Bonora E, Muggeo M.** Cause-specific mortality in type 2 diabetes. The Verona Diabetes Study. *Diabetes Care* 1999; **22**: 756-761 [PMID: 10332677 DOI: 10.2337/diacare.22.5.756]
- Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM.** Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 2002; **346**: 393-403 [PMID: 11832527 DOI: 10.1056/NEJMoa012512]
- Patel A, MacMahon S, Chalmers J, Neal B, Billot L, Woodward M, Marre M, Cooper M, Glasziou P, Grobbee D, Hamet P, Harrap S, Heller S, Liu L, Mancia G, Mogensen CE, Pan C, Poulter N, Rodgers A, Williams B, Bompont S, de Galan BE, Joshi R, Travert F.** Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. *N Engl J Med* 2008; **358**: 2560-2572 [PMID: 18539916 DOI: 10.1056/NEJMoa0802987]
- Gerstein HC, Miller ME, Byington RP, Goff DC, Bigger JT, Buse JB, Cushman WC, Genuth S, Ismail-Beigi F, Grimm RH, Probstfield JL, Simons-Morton DG, Friedewald WT.** Effects of intensive glucose lowering in type 2 diabetes. *N Engl J Med* 2008; **358**: 2545-2559 [PMID: 18539917 DOI: 10.1056/NEJMoa0802743]
- Tripathy D, Schwenke DC, Banerji M, Bray GA, Buchanan TA, Clement SC, Henry RR, Kitabchi AE, Mudaliar S, Ratner RE, Stentz FB, Masi N, Reaven PD, DeFronzo RA.** Diabetes Incidence and Glucose Tolerance after Termination of Pioglitazone Therapy: Results from ACT NOW. *J Clin Endocrinol Metab* 2016; **101**: 2056-2062 [PMID: 26982008 DOI: 10.1210/jc.2015-4202]
- Chiasson JL, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M.** Acarbose treatment and the risk of cardiovascular disease and hypertension in patients with impaired glucose tolerance: the STOP-NIDDM trial. *JAMA* 2003; **290**: 486-494 [PMID: 12876091 DOI: 10.1001/jama.290.4.486]
- Kernan WN, Viscoli CM, Furie KL, Young LH, Inzucchi SE, Gorman M, Guarino PD, Lovejoy AM, Peduzzi PN, Conwit R, Brass LM, Schwartz GG, Adams HP, Berger L, Carolei A, Clark W, Coull B, Ford GA, Kleindorfer D, O'Leary JR, Parsons MW, Ringleb P, Sen S, Spence JD, Tanne D, Wang D, Winder TR.** Pioglitazone after Ischemic Stroke or Transient Ischemic Attack. *N Engl J Med* 2016; **374**: 1321-1331 [PMID: 26886418 DOI: 10.1056/NEJMoa1506930]
- Selvin E, Steffes MW, Zhu H, Matsushita K, Wagenknecht L, Pankow J, Coresh J, Brancati FL.** Glycated hemoglobin, diabetes, and cardiovascular risk in nondiabetic adults. *N Engl J Med* 2010; **362**: 800-811 [PMID: 20200384 DOI: 10.1056/NEJMoa0908359]
- Nakagami T.** Hyperglycaemia and mortality from all causes and from cardiovascular disease in five populations of Asian origin. *Diabetologia* 2004; **47**: 385-394 [PMID: 14985967 DOI: 10.1007/s00125-004-1334-6]
- Qiao Q, Dekker JM, de Vegt F, Nijpels G, Nissinen A, Stehouwer CD, Bouter LM, Heine RJ, Tuomilehto J.** Two prospective studies found that elevated 2-hr glucose predicted male mortality independent of fasting glucose and HbA1c. *J Clin Epidemiol* 2004; **57**: 590-596 [PMID: 15246127 DOI: 10.1016/j.jclinepi.2003.10.007]
- Tuomilehto J, Lindström J, Eriksson JG, Valle TT, Hämäläinen H, Ilanne-Parikka P, Keinänen-Kiukkaanniemi S, Laakso M, Louheranta A, Rastas M, Salminen V, Uusitupa M.** Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N Engl J Med* 2001; **344**: 1343-1350 [PMID: 11333990 DOI: 10.1056/NEJM200105033441801]
- Ko GT, Chan JC, Woo J, Lau E, Yeung VT, Chow CC, Cockram CS.** The reproducibility and usefulness of the oral glucose tolerance test in screening for diabetes and other cardiovascular risk factors. *Ann Clin Biochem* 1998; **35** (Pt 1): 62-67 [PMID: 9463740]
- Barr RG, Nathan DM, Meigs JB, Singer DE.** Tests of glycemia for the diagnosis of type 2 diabetes mellitus. *Ann Intern Med* 2002; **137**: 263-272 [PMID: 12186517]
- Braga F, Dolci A, Mosca A, Panteghini M.** Biological variability of glycated hemoglobin. *Clin Chim Acta* 2010; **411**: 1606-1610 [PMID: 20688052 DOI: 10.1016/j.cca.2010.07.030]
- Morris DH, Khunti K, Achana F, Srinivasan B, Gray LJ, Davies MJ, Webb D.** Progression rates from HbA1c 6.0-6.4% and other prediabetes definitions to type 2 diabetes: a meta-analysis. *Diabetologia* 2013; **56**: 1489-1493 [PMID: 23584433 DOI: 10.1007/s00125-013-2902-4]
- Edelman D, Olsen MK, Dudley TK, Harris AC, Oddone EZ.** Utility of hemoglobin A1c in predicting diabetes risk. *J Gen Intern Med* 2004; **19**: 1175-1180 [PMID: 15610327 DOI: 10.1111/j.1525-1497.2004.40178.x]
- Inoue K, Matsumoto M, Kobayashi Y.** The combination of fasting plasma glucose and glycosylated hemoglobin predicts type 2 diabetes in Japanese workers. *Diabetes Res Clin Pract* 2007; **77**: 451-458 [PMID: 17346846 DOI: 10.1016/j.diabres.2007.01.024]
- Droumaguet C, Balkau B, Simon D, Caces E, Tichet J, Charles MA, Eschwege E.** Use of HbA1c in predicting progression to diabetes in French men and women: data from an Epidemiological Study on the Insulin Resistance Syndrome (DESIR). *Diabetes Care* 2006; **29**: 1619-1625 [PMID: 16801588 DOI: 10.2337/dc05-2525]
- Sato KK, Hayashi T, Harita N, Yoneda T, Nakamura Y, Endo G, Kambe H.** Combined measurement of fasting plasma glucose and A1C is effective for the prediction of type 2 diabetes: the Kansai Healthcare Study. *Diabetes Care* 2009; **32**: 644-646 [PMID: 19131461 DOI: 10.2337/dc08-1631]
- Cowie CC, Rust KF, Byrd-Holt DD, Gregg EW, Ford ES, Geiss LS, Bainbridge KE, Fradkin JE.** Prevalence of diabetes and high risk for diabetes using A1C criteria in the U.S. population in 1988-2006. *Diabetes Care* 2010; **33**: 562-568 [PMID: 20067953 DOI: 10.2337/dc09-1524]
- Heianza Y, Hara S, Arase Y, Saito K, Fujiwara K, Tsuji H, Kodama S, Hsieh SD, Mori Y, Shimano H, Yamada N, Kosaka K, Sone H.** HbA1c 5.7-6.4% and impaired fasting plasma glucose for diagnosis of prediabetes and risk of progression to diabetes in Japan (TOPICS 3): a longitudinal cohort study. *Lancet* 2011; **378**: 147-155 [PMID: 21705064 DOI: 10.1016/S0140-6736(11)60472-8]
- Mann DM, Carson AP, Shimbo D, Fonseca V, Fox CS, Muntner P.** Impact of A1C screening criterion on the diagnosis of pre-diabetes among U.S. adults. *Diabetes Care* 2010; **33**: 2190-2195 [PMID: 20628087 DOI: 10.2337/dc10-0752]
- Bullard KM, Saydah SH, Imperatore G, Cowie CC, Gregg EW, Geiss LS, Cheng YJ, Rolka DB, Williams DE, Caspersen CJ.** Secular changes in U.S. Prediabetes prevalence defined by hemoglobin A1c and fasting plasma glucose: National Health and Nutrition Examination Surveys, 1999-2010. *Diabetes Care* 2013; **36**: 2286-2293 [PMID: 23603918 DOI: 10.2337/dc12-2563]



- 28 **Rosella LC**, Lebenbaum M, Fitzpatrick T, Zuk A, Booth GL. Prevalence of Prediabetes and Undiagnosed Diabetes in Canada (2007-2011) According to Fasting Plasma Glucose and HbA1c Screening Criteria. *Diabetes Care* 2015; **38**: 1299-1305 [PMID: 25852207 DOI: 10.2337/dc14-2474]
- 29 **Mainous AG**, Tanner RJ, Baker R, Zayas CE, Harle CA. Prevalence of prediabetes in England from 2003 to 2011: population-based, cross-sectional study. *BMJ Open* 2014; **4**: e005002 [PMID: 24913327 DOI: 10.1136/bmjopen-2014-005002]
- 30 **Kumar A**, Wong R, Ottenbacher KJ, Al Snih S. Prediabetes, undiagnosed diabetes, and diabetes among Mexican adults: findings from the Mexican Health and Aging Study. *Ann Epidemiol* 2016; **26**: 163-170 [PMID: 26872919 DOI: 10.1016/j.annepidem.2015.12.006]
- 31 **Guerrero-Romero F**, Rodríguez-Morán M, Pérez-Fuentes R, Sánchez-Guillén MC, González-Ortiz M, Martínez-Abundis E, Brito-Zurita O, Madero A, Figueroa B, Revilla-Monsalve C, Flores-Martínez SE, Islas-Andrade S, Rascón-Pacheco RA, Cruz M, Sánchez-Corona J. Prediabetes and its relationship with obesity in Mexican adults: The Mexican Diabetes Prevention (MexDiab) Study. *Metab Syndr Relat Disord* 2008; **6**: 15-23 [PMID: 18370832 DOI: 10.1089/met.2007.0020]
- 32 **Di Pino A**, Urbano F, Zagami RM, Filippello A, Di Mauro S, Piro S, Purrello F, Rabuazzo AM. Low Endogenous Secretory Receptor for Advanced Glycation End-Products Levels Are Associated With Inflammation and Carotid Atherosclerosis in Prediabetes. *J Clin Endocrinol Metab* 2016; **101**: 1701-1709 [PMID: 26885882 DOI: 10.1210/jc.2015-4069]
- 33 **Unwin N**, Shaw J, Zimmet P, Alberti KG. Impaired glucose tolerance and impaired fasting glycaemia: the current status on definition and intervention. *Diabet Med* 2002; **19**: 708-723 [PMID: 12207806]
- 34 **Abdul-Ghani MA**, Williams K, DeFronzo R, Stern M. Risk of progression to type 2 diabetes based on relationship between postload plasma glucose and fasting plasma glucose. *Diabetes Care* 2006; **29**: 1613-1618 [PMID: 16801587 DOI: 10.2337/dc05-1711]
- 35 **American Diabetes Association**. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2010; **33** Suppl 1: S62-S69 [PMID: 20042775 DOI: 10.2337/dc10-S062]
- 36 **Furukawa S**, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, Shimomura I. Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest* 2004; **114**: 1752-1761 [PMID: 15599400 DOI: 10.1172/JCI200421625.1752]
- 37 **Li J**, Ma H, Na L, Jiang S, Lv L, Li G, Zhang W, Na G, Li Y, Sun C. Increased hemoglobin A1c threshold for prediabetes remarkably improving the agreement between A1c and oral glucose tolerance test criteria in obese population. *J Clin Endocrinol Metab* 2015; **100**: 1997-2005 [PMID: 25751104 DOI: 10.1210/jc.2014-4139]
- 38 **Yan ST**, Xiao HY, Tian H, Li CL, Fang FS, Li XY, Cheng XL, Li N, Miao XY, Yang Y, Wang LC, Zou XM, Ma FL, He Y, Sai XY. The cutoffs and performance of glycated hemoglobin for diagnosing diabetes and prediabetes in a young and middle-aged population and in an elderly population. *Diabetes Res Clin Pract* 2015; **109**: 238-245 [PMID: 26059072 DOI: 10.1016/j.diabres.2015.05.047]
- 39 **Shimodaira M**, Okaniwa S, Hanyu N, Nakayama T. Optimal Hemoglobin A1c Levels for Screening of Diabetes and Prediabetes in the Japanese Population. *J Diabetes Res* 2015; **2015**: 932057 [PMID: 26114121 DOI: 10.1155/2015/932057]
- 40 **McCance DR**, Hanson RL, Charles MA, Jacobsson LT, Pettitt DJ, Bennett PH, Knowler WC. Comparison of tests for glycated haemoglobin and fasting and two hour plasma glucose concentrations as diagnostic methods for diabetes. *BMJ* 1994; **308**: 1323-1328 [PMID: 8019217 DOI: 10.1136/bmj.308.6940.1323]
- 41 **Colagiuri S**, Davies D. The value of early detection of type 2 diabetes. *Curr Opin Endocrinol Diabetes Obes* 2009; **16**: 95-99 [PMID: 19276801 DOI: 10.1097/MED.0b013e328329302f]
- 42 **Khaw KT**, Wareham N, Bingham S, Luben R, Welch A, Day N. Association of hemoglobin A1c with cardiovascular disease and mortality in adults: the European prospective investigation into cancer in Norfolk. *Ann Intern Med* 2004; **141**: 413-420 [PMID: 15381514]
- 43 **Di Pino A**, Scicali R, Calanna S, Urbano F, Mantegna C, Rabuazzo AM, Purrello F, Piro S. Cardiovascular risk profile in subjects with prediabetes and new-onset type 2 diabetes identified by HbA(1c) according to American Diabetes Association criteria. *Diabetes Care* 2014; **37**: 1447-1453 [PMID: 24574348 DOI: 10.2337/dc13-2357]
- 44 **Jørgensen L**, Jenssen T, Joakimsen O, Heuch I, Ingebrechtsen OC, Jacobsen BK. Glycated hemoglobin level is strongly related to the prevalence of carotid artery plaques with high echogenicity in nondiabetic individuals: the Tromsø study. *Circulation* 2004; **110**: 466-470 [PMID: 15249512 DOI: 10.1161/01.CIR.0000136809.55141.3B]
- 45 **Carson AP**, Steffes MW, Carr JJ, Kim Y, Gross MD, Carnethon MR, Reis JP, Loria CM, Jacobs DR, Lewis CE. Hemoglobin a1c and the progression of coronary artery calcification among adults without diabetes. *Diabetes Care* 2015; **38**: 66-71 [PMID: 25325881 DOI: 10.2337/dc14-0360]
- 46 **Aggarwal B**, Shah GK, Randhawa M, Ellis SG, Lincoff AM, Menon V. Utility of Glycated Hemoglobin for Assessment of Glucose Metabolism in Patients With ST-Segment Elevation Myocardial Infarction. *Am J Cardiol* 2016; **117**: 749-753 [DOI: 10.1016/j.amjcard.2015.11.060]
- 47 **Basta G**, Sironi AM, Lazzerini G, Del Turco S, Buzzigoli E, Casolaro A, Natali A, Ferrannini E, Gastaldelli A. Circulating soluble receptor for advanced glycation end products is inversely associated with glycemic control and S100A12 protein. *J Clin Endocrinol Metab* 2006; **91**: 4628-4634 [PMID: 16926247 DOI: 10.1210/jc.2005-2559]
- 48 **Koyama H**, Yamamoto H, Nishizawa Y. RAGE and soluble RAGE: potential therapeutic targets for cardiovascular diseases. *Mol Med* 2007; **13**: 625-635 [PMID: 17932553 DOI: 10.2119/2007-00087.Koyama]
- 49 **Calanna S**, Scicali R, Di Pino A, Knop FK, Piro S, Rabuazzo AM, Purrello F. Alpha- and beta-cell abnormalities in haemoglobin A1c-defined prediabetes and type 2 diabetes. *Acta Diabetol* 2014; **51**: 567-575 [PMID: 24442427 DOI: 10.1007/s00592-014-0555-5]
- 50 **Calanna S**, Scicali R, Di Pino A, Knop FK, Piro S, Rabuazzo AM, Purrello F. Lipid and liver abnormalities in haemoglobin A1c-defined prediabetes and type 2 diabetes. *Nutr Metab Cardiovasc Dis* 2014; **24**: 670-676 [DOI: 10.1016/j.numecd.2014.01.013]
- 51 **Zagami RM**, Di Pino A, Urbano F, Piro S, Purrello F, Rabuazzo AM. Low circulating Vitamin D levels are associated with increased arterial stiffness in prediabetic subjects identified according to HbA1c. *Atherosclerosis* 2015; **243**: 395-401 [DOI: 10.1016/j.atherosclerosis.2015.09.038]
- 52 **Schöttker B**, Rathmann W, Herder C, Thorand B, Wilsgaard T, Njølstad I, Siganos G, Mathiesen EB, Saum KU, Peasey A, Feskens E, Boffetta P, Trichopoulou A, Kuulasmaa K, Kee F, Brenner H. HbA1c levels in non-diabetic older adults - No J-shaped associations with primary cardiovascular events, cardiovascular and all-cause mortality after adjustment for confounders in a meta-analysis of individual participant data from six cohort studies. *BMC Med* 2016; **14**: 26 [DOI: 10.1186/s12916-016-0570-1]
- 53 **Wilcox R**, Bousser MG, Betteridge DJ, Scherthaner G, Pirags V, Kupfer S, Dormandy J. Effects of pioglitazone in patients with type 2 diabetes with or without previous stroke: results from PROactive (PROspective pioglitazone Clinical Trial In macroVascular Events 04). *Stroke* 2007; **38**: 865-873 [PMID: 17290029 DOI: 10.1161/01.STR.0000257974.06317.49]
- 54 **Frye RL**, August P, Brooks MM, Hardison RM, Kelsey SF, MacGregor JM, Orchard TJ, Chaitman BR, Genuth SM, Goldberg SH, Hlatky MA, Jones TL, Molitch ME, Nesto RW, Sako EY, Sobel BE. A randomized trial of therapies for type 2 diabetes and coronary artery disease. *N Engl J Med* 2009; **360**: 2503-2515 [PMID: 19502645 DOI: 10.1056/NEJMoa0805796]
- 55 **Hopper I**, Billah B, Skiba M, Krum H. Prevention of diabetes and reduction in major cardiovascular events in studies of subjects with prediabetes: meta-analysis of randomised controlled clinical trials. *Eur J Cardiovasc Prev Rehabil* 2011; **18**: 813-823 [PMID: 21878448 DOI: 10.1177/1741826711421687]
- 56 **Bobbert T**, Mai K, Fischer-Rosinsky A, Pfeiffer AF, Spranger J.



- A1C is associated with intima-media thickness in individuals with normal glucose tolerance. *Diabetes Care* 2010; **33**: 203-204 [PMID: 19808917 DOI: 10.2337/dc09-1009]
- 57 **Abdul-Ghani MA**, Abdul-Ghani T, Ali N, DeFronzo RA. One-hour plasma glucose concentration and the metabolic syndrome identify subjects at high risk for future type 2 diabetes. *Diabetes Care* 2008; **31**: 1650-1655 [PMID: 18487478]
  - 58 **Fiorentino TV**, Marini MA, Andreozzi F, Arturi F, Succurro E, Perticone M, Sciacqua A, Hribal ML, Perticone F, Sesti G. One-Hour Postload Hyperglycemia Is a Stronger Predictor of Type 2 Diabetes Than Impaired Fasting Glucose. *J Clin Endocrinol Metab* 2015; **100**: 3744-3751 [PMID: 26274345 DOI: 10.1210/jc.2015-2573]
  - 59 **Succurro E**, Marini MA, Arturi F, Grembale A, Lugarà M, Andreozzi F, Sciacqua A, Lauro R, Hribal ML, Perticone F, Sesti G. Elevated one-hour post-load plasma glucose levels identifies subjects with normal glucose tolerance but early carotid atherosclerosis. *Atherosclerosis* 2009; **207**: 245-249 [PMID: 19410252 DOI: 10.1016/j.atherosclerosis.2009.04.006]
  - 60 **Succurro E**, Arturi F, Lugarà M, Grembale A, Fiorentino TV, Caruso V, Andreozzi F, Sciacqua A, Hribal ML, Perticone F, Sesti G. One-hour postload plasma glucose levels are associated with kidney dysfunction. *Clin J Am Soc Nephrol* 2010; **5**: 1922-1927 [PMID: 20595688 DOI: 10.2215/CJN.03240410]
  - 61 **Abdul-Ghani MA**, Lyssenko V, Tuomi T, DeFronzo RA, Groop L. Fasting versus postload plasma glucose concentration and the risk for future type 2 diabetes: results from the Botnia Study. *Diabetes Care* 2009; **32**: 281-286 [PMID: 19017778 DOI: 10.2337/dc08-1264]
  - 62 **Priya M**, Anjana RM, Chiwanga FS, Gokulakrishnan K, Deepa M, Mohan V. 1-hour venous plasma glucose and incident prediabetes and diabetes in Asian Indians. *Diabetes Technol Ther* 2013; **15**: 497-502 [PMID: 23550555 DOI: 10.1089/dia.2013.0025]
  - 63 **DeFronzo RA**. The triumvirate:  $\beta$ -cell, muscle, liver. A collusion responsible for NIDDM. *Diabetes* 1988; **37**: 667-87 [PMID: 3289989 DOI: 10.2337/diab.37.6.667]
  - 64 **Sciacqua A**, Miceli S, Carullo G, Greco L, Succurro E, Arturi F, Sesti G, Perticone F. One-hour postload plasma glucose levels and left ventricular mass in hypertensive patients. *Diabetes Care* 2011; **34**: 1406-1411 [PMID: 21515837 DOI: 10.2337/dc11-0155]
  - 65 **Lee JE**. Alternative biomarkers for assessing glycemic control in diabetes: fructosamine, glycated albumin, and 1,5-anhydroglucitol. *Ann Pediatr Endocrinol Metab* 2015; **20**: 74-78 [PMID: 26191510 DOI: 10.6065/apem.2015.20.2.74]
  - 66 **Ikezaki H**, Furusyo N, Ihara T, Hayashi T, Ura K, Hiramine S, Mitumoto F, Takayama K, Murata M, Kohzuma T, Ai M, Schaefer EJ, Hayashi J. Glycated albumin as a diagnostic tool for diabetes in a general Japanese population. *Metabolism* 2015; **64**: 698-705 [PMID: 25817605 DOI: 10.1016/j.metabol.2015.03.003]
  - 67 **Juraschek SP**, Steffes MW, Miller ER, Selvin E. Alternative markers of hyperglycemia and risk of diabetes. *Diabetes Care* 2012; **35**: 2265-2270 [PMID: 22875225 DOI: 10.2337/dc12-0787]
  - 68 **Sumner AE**, Duong MT, Aldana PC, Ricks M, Tulloch-reid MK, Lozier JN, Chung ST, Sacks DB. A1C Combined With Glycated Albumin Improves Detection of Prediabetes in Africans. *Afri Ame Study* 2015; **1**: 1-7 [DOI: 10.2337/dc15-1699]
  - 69 **Selvin E**, Rawlings AM, Grams M, Klein R, Sharrett AR, Steffes M, Coresh J. Fructosamine and glycated albumin for risk stratification and prediction of incident diabetes and microvascular complications: a prospective cohort analysis of the Atherosclerosis Risk in Communities (ARIC) study. *Lancet Diabetes Endocrinol* 2014; **2**: 279-288 [PMID: 24703046 DOI: 10.1016/S2213-8587(13)70199-2]
  - 70 **Lu L**, Pu LJ, Xu XW, Zhang Q, Zhang RY, Zhang JS, Hu J, Yang ZK, Lu AK, Ding FH, Shen J, Chen QJ, Lou S, Fang DH, Shen WF. Association of serum levels of glycated albumin, C-reactive protein and tumor necrosis factor- $\alpha$  with the severity of coronary artery disease and renal impairment in patients with type 2 diabetes mellitus. *Clin Biochem* 2007; **40**: 810-816 [PMID: 17499233 DOI: 10.1016/j.clinbiochem.2007.03.022]
  - 71 **Kim WJ**, Park CY, Park SE, Rhee EJ, Lee WY, Oh KW, Park SW, Kim SW, Park HS, Kim YJ, Song SJ, Ahn HY. Serum 1,5-anhydroglucitol is associated with diabetic retinopathy in Type 2 diabetes. *Diabet Med* 2012; **29**: 1184-1190 [PMID: 22332964 DOI: 10.1111/j.1464-5491.2012.03613.x]
  - 72 **Watanabe K**, Suzuki T, Ouchi M, Suzuki K, Ohara M, Hashimoto M, Yamashita H, Okazaki M, Ishii K, Oba K. Relationship between postprandial glucose level and carotid artery stiffness in patients without diabetes or cardiovascular disease. *BMC Cardiovasc Disord* 2013; **13**: 11 [PMID: 23442745 DOI: 10.1186/1471-2261-13-11]
  - 73 **Selvin E**, Rawlings A, Lutsey P, Maruthur N, Pankow JS, Steffes M, Coresh J. Association of 1,5-Anhydroglucitol With Cardiovascular Disease and Mortality. *Diabetes* 2016; **65**: 201-208 [PMID: 26395741 DOI: 10.2337/db15-0607]

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## Nitrate-nitrite-nitrosamines exposure and the risk of type 1 diabetes: A review of current data

Zahra Bahadoran, Asghar Ghasemi, Parvin Mirmiran, Fereidoun Azizi, Farzad Hadaegh

Zahra Bahadoran, Parvin Mirmiran, Nutrition and Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran 19395-4763, Iran

Asghar Ghasemi, Endocrine Physiology Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran 19395-4763, Iran

Fereidoun Azizi, Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran 19395-4763, Iran

Farzad Hadaegh, Prevention of Metabolic Disorders Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran 19395-4763, Iran

**Author contributions:** Bahadoran Z, Ghasemi A and Hadaegh F prepared the manuscript; Bahadoran Z, Mirmiran P and Hadaegh F designed and implemented the study; Mirmiran P, Azizi F and Hadaegh F revised and supervised overall project; all authors read and approved the final version of manuscript.

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**Correspondence to:** Parvin Mirmiran, PhD, Nutrition and Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, No. 24, Sahid-Erabi St, Yemen St, Chamran Exp, Tehran 19395-4763, Iran. [mirmiran@endocrine.ac.ir](mailto:mirmiran@endocrine.ac.ir)  
 Telephone: +98-21-22432500  
 Fax: +98-21-22416264

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### Abstract

The potential toxic effects of nitrate-nitrite-nitrosamine on pancreatic  $\beta$  cell have remained a controversial issue over the past two decades. In this study, we reviewed epidemiological studies investigated the associations between nitrate-nitrite-nitrosamines exposure, from both diet and drinking water to ascertain whether these compounds may contribute to development of type 1 diabetes. To identify relevant studies, a systematic search strategy of PubMed, Scopus, and Science Direct was conducted using queries including the key words "nitrate", "nitrite", "nitrosamine" with "type 1 diabetes" or "insulin dependent diabetes mellitus". All searches were limited to studies published in English. Ecologic surveys, case-control and cohort studies have indicated conflicting results in relation to nitrate-nitrite exposure from drinking water and the risk of type 1 diabetes. A null, sometimes even negative association has been mainly reported in regions with a mean nitrate levels < 25 mg/L in drinking water, while increased risk of type 1 diabetes was observed in those with a maximum nitrate levels > 40-80 mg/L. Limited data are available regarding the potential diabetogenic effect of nitrite from drinking water, although there is evidence indicating dietary nitrite could be a risk factor for development of type 1 diabetes, an effect however that seems to be significant in a higher range of acceptable limit for nitrate/nitrite. Current data regarding dietary exposure of nitrosamine and development of type 1 diabetes is also inconsistent. Considering to an increasing trend of type 1 diabetes mellitus (T1DM) along with an elevated nitrate-nitrite exposure, additional research is critical to clarify potential harmful effects of nitrate-nitrite-nitrosamine exposure on  $\beta$ -cell autoimmunity and the risk of T1DM.

**Key words:** Nitrate; Nitrite; Nitrosamine; Type 1 diabetes

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**Core tip:** The potential toxic effects of nitrate-nitrite-nitrosamine on pancreatic  $\beta$  cell have remained a controversial issue over the past two decades. Ecologic surveys, case-control and cohort studies have indicated conflicting results in relation to nitrate-nitrite exposure from drinking water and the risk of type 1 diabetes. An increased risk of type 1 diabetes was observed in regions with a maximum nitrate levels > 40-80 mg/L. Dietary nitrite could be a risk for development of type 1 diabetes in a higher range of acceptable limit. Additional research is critical to clarify potential harmful effects of nitrate-nitrite-nitrosamine exposure on  $\beta$ -cell autoimmunity and the risk of type 1 diabetes mellitus.

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## INTRODUCTION

### An overview of type 1 diabetes

Type 1 diabetes mellitus (T1DM), one of the main autoimmune disorders caused by immune-mediated destruction of pancreatic  $\beta$ -cells, eventually develops to an absolute insulin deficiency, impaired glucose homeostasis, and physiological dependence on exogenous insulin<sup>[1]</sup>. An overall approximately 3% increased per year in the incidence of T1DM along with a different geographical incidence has been observed worldwide<sup>[2,3]</sup>. A higher incidence rate of T1DM has been reported in European countries, especially Finland and Sardinia, however recently the incidence of T1DM has risen rapidly in low-incident populations including parts of India, the Middle East, and Sub-Saharan Africa<sup>[4]</sup>. The incidence of T1DM had an increasing trend in both developed and developing countries during a recent decade<sup>[5-8]</sup>. According to current trends, it is predicted that new cases of T1DM in European children < 5 year will be doubled and prevalent cases < 15 years will be raised by 70%, between 2005 and 2020<sup>[7]</sup>.

### Risk factors of T1DM

T1DM has a multifactorial nature; besides genetic factors and family history which account for about 30%-50% onset of T1DM, several factors such as environmental contaminants, infection agents, drugs, and dietary factors have been known as important etiologically relevant to  $\beta$ -cell autoimmunity and T1DM development<sup>[9-11]</sup>. Dietary factors implicated in the pathogenesis of T1DM are cow milk proteins (casein, bovine serum albumin,  $\beta$ -lactoglobulin, and bovine insulin), gluten, zinc and

vitamin D deficiency, as well as nitrate and nitrite; some ecologic, animal, and human studies have confirmed that *N*-nitroso compounds, nitrate and nitrite play a role in development of T1DM<sup>[12-14]</sup>. Diabetogenic agents from diet may induce their effects by several suggested pathways, including apoptosis of  $\beta$ -cell, increased oxidative stress, impaired insulin response and immune function, and some postprandial modifications<sup>[15]</sup>.

### Diabetogenic hypothesis of nitrate-nitrite exposure

Inorganic nitrate ( $\text{NO}_3$ ) and nitrite ( $\text{NO}_2$ ) are naturally occurring compounds in foods and are also used as food additives; major sources of exogenous nitrate exposure are vegetables and drinking water, whereas processed meat and animal food products are the main sources of nitrite<sup>[16]</sup>. Considering both acute and chronic potential toxicities, some limitations have been legislated for dietary intakes of nitrate and nitrite; the acceptable daily intakes (ADI) of nitrate and nitrite from food sources as designated by the Scientific Committee on Foods and the Joint Food and Agriculture Organization/World Health Organization (WHO) Expert Committee on Food Additives defined as 3.7 and 0.06 mg/kg body weight, respectively<sup>[17]</sup>. Moreover, due to substantial concentrations of nitrate-nitrite in drinking water, WHO restricted the acceptable concentrations of drinking water to < 50 mg/L and 3 mg/L for nitrate and nitrite, respectively<sup>[18,19]</sup>.

Recent investigations have however highlighted the beneficial therapeutic effects of nitrate-nitrite against metabolic disorders such as type 2 diabetes<sup>[20-22]</sup>, possible adverse complications such as thyroid disorders and T1DM<sup>[23,24]</sup> are still remaining due to indiscriminate increased use of fertilizers and nitrite-containing food additives and increased exposure of nitrate-nitrite from both diet and drinking water. It has been proposed that nitrate-nitrite may have toxic effects on pancreatic  $\beta$ -cells due to generation of peroxynitrite, reactive nitrogen intermediates, and nitrosamines<sup>[25]</sup>.

Although data have shown elevated risk of  $\beta$ -cell autoimmunity and T1DM due to high intakes of nitrate-nitrite over the past two decades<sup>[26]</sup>, this data has however not yet led to a consistent confirmed conclusion<sup>[27-30]</sup>.

### Aim of this study

This review will focus on the potential effect of nitrate-nitrite-nitrosamines exposure on development of T1DM. We reviewed epidemiological studies investigating the associations between nitrate-nitrite-nitrosamines exposure, from both diet and drinking water to ascertain whether higher nitrate-nitrite may contribute to the development of T1DM.

To identify relevant studies, a systematic search strategy of PubMed, Scopus, and Science Direct was conducted using queries including the key words "nitrate", "nitrite", "nitrosamine" with "T1DM" or "insulin dependent diabetes mellitus". All searches were limited to studies published in English.

## EXPOSURE OF NITRATE-NITRITE FROM DRINKING WATER AND THE RISK OF T1DM

Possible relation between quality of drinking water and T1DM, particularly concentrations of nitrate or nitrite has been investigated in several studies. An overview of current data indicates that potential diabetogenic effects of nitrate-nitrite have mainly been investigated by estimation of nitrate-nitrite exposure from drinking water, a relationship also evaluated in the framework of ecological studies<sup>[28-32]</sup>.

An ecological analysis of insulin dependent diabetes mellitus registry data on children aged < 18 year during 1978-1988 in relation to public water supplies and well water systems in Colorado between 1984 and 1988, showed a significant correlation between T1DM incidence and water nitrate level ( $r = 0.27$ ,  $P = 0.03$ )<sup>[29]</sup>. This correlation was higher in countries where nitrate levels in the public water system were in the highest tertile ( $r = 0.29$ ,  $P = 0.02$ ), and the rate of T1DM was higher in the highest compared to the lowest tertile of nitrate exposure (PI = 15/100000 vs PI = 7/100000, in 0.77-8.2 mg/L vs 0-0.08 mg/L nitrate levels, model  $R^2 = 0.14$ ); the authors pointed out that findings of the study should be interpreted considering to some limitations such as lack of data on individual's nitrate exposure and inappropriate timing of the exposure measurement<sup>[29]</sup>.

In another ecological analysis, conducted on a population-based study in the framework of Yorkshire Regional Health Authority during 1978-1994, Parslow *et al.*<sup>[28]</sup> reported that the incidence of T1DM was positively associated with mean nitrate levels in drinking water; an increasing trend in standardized incidence ratio (SIR) of T1DM was observed across increasing levels of nitrate in drinking water (SIR = 85, 95%CI: 78-93; SIR = 99, 95%CI: 91-107; SIR = 115, 95%CI: 107-124; in levels of 1.5-3.2, 3.2-14.5 and 14.9-40.0 mg/L, respectively,  $\chi^2 = 26.8$ ,  $P < 0.001$ )<sup>[28]</sup>. Moreover, a 30% higher incidence rate of diabetes was observed among doses in water supply zones with mean nitrate levels 14.9-40.0 mg/L, compared with those in zones with a mean nitrate levels < 3.2 mg/L (IRR = 1.27, 95%CI: 1.09-1.48). In this study, over 30% of drinking water samples contained > 25 mg/L nitrate levels.

Analysis of drinking water in Finland for nitrate, nitrite, nitrate-nitrogen and nitrite-nitrogen, among families with a child, diagnosed as type 1 diabetic compared to controls, showed that higher levels of nitrate in drinking water was related to increased risk of T1DM (OR = 1.32, 95%CI: 1.06-1.64;  $P = 0.013$ ); nitrite concentrations had no significant association with the risk of T1DM (OR = 0.36, 95%CI: 0.06-2.03;  $P = 0.25$ )<sup>[33]</sup>. Mean nitrate and nitrite levels of drinking water in this population were 4.43 (0-80 mg/L) and 0.02 (0.02-0.16 mg/L), respectively; mean nitrate and nitrite levels in municipalities with high compared to low incidence of T1DM, was lower (1.27 mg/L vs 3.25 mg/L, 0.02 mg/L vs 0.03 mg/L, for nitrate

and nitrite, respectively)<sup>[33]</sup>.

In contrast, some studies report findings to reject diabetogenic hypothesis of the nitrate-nitrite exposure. The incidence of T1DM was not related to nitrate exposure, in an ecological study of children, aged < 15 year, in the Netherlands, conducted using the Dutch Pediatric Surveillance Unit (1993-1995) and nitrate drinking water data from the National Institute of Public Health and Environmental Protection (1991-1995)<sup>[31]</sup>; standardized incidence rate of T1DM was 1.45 for nitrate levels ranging > 25 mg/L (95%CI: 0.85-2.07). Lack of information on the individual's quantity of water consumption, length of exposure and data on potential risk factors of such as family history of T1DM, were important limitations of this study. Moreover, non-significant findings have been attributed to small number of cases in the > 25 mg/L category; in this study, only 1% of the children were exposed to nitrate levels between 25-41 mg/L<sup>[31]</sup>.

In a Finnish nation-wide case-control study, exposure of nitrate and nitrite in children and their parents from drinking water were assessed in relation to risk of T1DM; no differences were observed in intakes of nitrate or nitrite from drinking water between cases and controls<sup>[26]</sup>.

Analysis of data on nitrate concentration of both tap and bottled water in Italy during 1993-1994, showed no significant association between nitrate exposure and incidence of T1DM during 1989-1998, in the subjects, aged 0-14 y ( $r = -0.06$ ) or in the group, aged 0-29 year ( $r = -0.17$ )<sup>[32]</sup>. There was no effect from sex in the same age-groups; in contrast with previous reports, a negative trend between nitrate levels and T1DM was also noted<sup>[32]</sup>; in this study, both tap and bottled water were within the acceptable maximal concentration of 50 mg/L legislated by the European Community and also under the recommended levels of 25 mg/L.

In a retrospective study of 153 Sardinian communes, among 0-14 year Italian children, a significantly inverse trend between childhood diabetes and mean nitrate exposure was observed; higher nitrate of drinking water was reported in districts with low compared to high incidence of T1DM (8.9-14.5 mg/L vs 4.3-7.8 mg/L)<sup>[34]</sup>. The risk of T1DM in subjects exposed to highest compared to the lowest nitrate levels in drinking water (6.5-28.9 mg/L vs  $\leq 2.5$  mg/L) decreased 40% (RR = 0.6, 95%CI: 0.4-1.0,  $P = 0.027$ ).

An initial assessment of nitrate exposure from domestic water during 1993-1997, in relation to T1DM diagnosed in children aged 0-15 year in England between 1975-1996, suggested that nitrate may had a protective effects against development of T1DM<sup>[35]</sup>; standardized incidence ratio in the highest compared to the lowest tertile of nitrate levels (7.48-16.58 mg/L vs 1-3.65 mg/L) was lower (SIR = 90.2, 95%CI: 77-105 vs SIR = 111.8, 95%CI: 96-129;  $\chi^2 = 3.89$ ,  $P = 0.048$ ), however, Poisson regression analysis failed to support this relationship; mean nitrate levels of drinking water was 6.02 mg/L (min = 0.48 and max = 31.9 mg/L).

Moltchanova *et al.*<sup>[36]</sup> in a study of children aged < 15 in Finland between 1987-1996, showed an increasing



risk of T1DM along with increasing nitrate concentration of drinking water. The posterior mean unit effect of nitrate on diabetes risk was 0.003 (-0.009, 0.0138), *i.e.*, 1 mg/L increased nitrate concentration in the ground water resulted in 0.3% increased risk of T1DM; mean nitrate level of groundwater was 6.22 mg/L (0.20 and 6.64 in the 1<sup>st</sup> and 4<sup>th</sup> quartiles, respectively)<sup>[36]</sup>.

In a retrospective study from Saudi Arab, type 1 diabetic patients, diagnosed between 1980 and 2009, no etiological effects for nitrate levels in drinking water of the study areas were observed; mean nitrate level in drinking water showed levels between 0.6 and 4 mg/L, during 30 years which were much lower than the toxic levels<sup>[37]</sup>.

A nested case-control analysis on 95 islet auto-antibody-positive (Islet Ab<sup>+</sup>) and 139 Islet Ab<sup>-</sup> children, conducted in the framework of German BABYDIAB study, indicated no association between nitrate content of drinking water and the risk of islet autoimmunity, whereas higher levels of nitrite ( $\geq 0.009$  mg/L vs  $< 0.009$  mg/L) had a borderline protective effect (OR = 0.6, 95%CI: 0.4-1.0)<sup>[30]</sup>; mean nitrate levels of water were 9.5 mg/L (4.8-16.6) and 9.2 mg/L (3.8-21.2) in Islet Ab<sup>+</sup> and Islet Ab<sup>-</sup> children, respectively; upper nitrite level of drinking water was marginally higher in Islet Ab<sup>-</sup> compared to Islet Ab<sup>+</sup> children (0.01 mg/L vs 0.009 mg/L,  $P = 0.06$ ). The odds of the progression of islet autoimmunity to T1DM in higher levels of nitrate and nitrite in drinking water ( $\geq 9.58$  and  $\geq 0.009$  mg/L) was 0.9 (95%CI: 0.4-2.0) and 1.5 (95%CI: 0.6-3.5), respectively<sup>[30]</sup>. Another important finding was an inverse relation between nitrate concentrations and pH levels of drinking water ( $r = -0.28$ ,  $P = 0.001$ ), along with a positive relation between pH of water and progression of T1DM (OR = 2.5, 95%CI: 1.1-5.7)<sup>[30]</sup>; it may be indirectly provide evidence for hazardous effects of nitrate on T1DM. This study was the first try to investigate the association of nitrate-nitrite exposure during the first year of life in children and the risk of islet autoimmunity; due to importance of this period in developing of islet autoimmunity, this study provided an opportunity to evaluate a potential causal relationship between nitrate-nitrite of drinking water and T1DM progression. Matching for date of birth, duration of follow-up, human leukocyte antigen (HLA), gender and geographical region and also adjustment of main potential risk factors of T1DM including genetic factors (HLA DR 3/4, 4/4) and maternal T1DM were other strengths of the study.

Tables 1 and 2 provide a summary of results from ecological, case-control and cohort studies of mean nitrate-nitrite levels from drinking water in relation to incidence of T1DM.

## DIETARY EXPOSURE OF NITRATE-NITRITE AND THE RISK OF T1DM

The risk of T1DM in response to nitrate-nitrite exposure from diet has been evaluated in a limited number of

studies. In a prospective case-control study of Swedish children, aged 0-14 years, matched for age, sex, and country of residence, a significant increasing trend of T1DM was noted for higher intakes of foods containing nitrate and nitrite<sup>[27]</sup>. In this study, fresh green vegetables, boiled vegetables, root vegetables, cheese, sausage and bacon have been defined as high nitrate-nitrite containing foods; mean frequency of nitrate-nitrite rich foods was higher in diabetics compared to controls; highest compared to the lowest ( $> 75^{\text{th}}$  centile vs  $< 25^{\text{th}}$  centile) frequency of consumption of nitrate-nitrite rich foods was related to an elevated risk of T1DM (OR = 2.41, 95%CI: 1.64-3.54,  $P = 0.001$ )<sup>[27]</sup>. After adjustment of some potential confounding variables including age, sex, maternal age, maternal education, and family history insulin dependent diabetes, the chance of having T1DM was 0.89 and 2.68 in individuals with medium and high nitrate-nitrite exposure from diet<sup>[27]</sup>. In further analysis, stratified for vitamin C rich foods, risk estimate for medium and highest nitrate-nitrite intakes along with low vitamin C intakes was 0.94 and 2.44 ( $P < 0.001$ ), respectively; in contrast, higher intakes of nitrate-nitrite were not associated with T1DM in the presence of higher intakes of vitamin C. An indirect estimation of nitrate-nitrite based on food frequency intakes, was an important limitation of this study; lack of data on nitrate-nitrite exposure from drinking water was also another source of bias in estimation of nitrate and nitrite exposure.

In a Finnish nation-wide case-control study, intakes of nitrate and nitrite of children and their parents from food and drinking water were assessed in relation to risk of T1DM<sup>[26]</sup>. Compared to controls, dietary intakes of nitrite were higher in diabetic children and their mothers (0.9 mg/d vs 0.8 mg/d). Higher intakes of nitrate were also observed in cases mother's compared to controls ( $P < 0.05$ ). The risk of T1DM increased across increasing intakes of dietary nitrite among children (OR = 1.16, 95%CI: 0.82-1.65; OR = 1.49, 95%CI: 1.06-2.10; OR = 2.32, 95%CI: 1.67-3.24 in the second, third, and fourth quartiles, respectively), and their mothers (OR = 1.15, 95%CI: 0.76-1.74; OR = 1.29, 95%CI: 0.87-1.91; OR = 1.98, 95%CI: 1.35-2.90, in the second, third, and fourth quartiles, respectively), a relationship independent of age, mother's education, place of residence or smoking status of mothers<sup>[26]</sup>.

A case-control study on dietary intakes of nitrate and nitrite during the year prior to diagnosis of diabetes, after adjustment of age, sex, and total energy intake, showed a non-significant positive dose-response relationship between risk of T1DM and nitrate intakes from foods (OR = 1; OR = 1.01, 95%CI: 0.028-3.61; OR = 1.19, 95%CI: 0.31-4.52, OR = 2.25, 95%CI: 0.45-11.14 in the first to fourth quartiles;  $P = 0.29$ ); dietary intakes of nitrate were  $< 5.66$ , 5.66-7.27, 7.27-9.01, and  $\geq 9.01$  mg/d in the first to fourth quartiles, respectively. The risk of T1DM increased 30% (OR = 1.30, 95%CI: 0.30-5.59) in the highest, compared to the lowest quartiles of nitrite intakes ( $\geq 4.82$  mg/d vs  $< 1.83$  mg/d)<sup>[38]</sup>. Neither were total intakes of nitrate + nitrite (from both diet and

**Table 1** Summary of results from ecological, case-control and cohort studies of mean nitrate levels from drinking water in relation to incidence of type 1 diabetes

Ref.	Country	Exposure levels (mg/L)	Findings
Muntoni <i>et al</i> <sup>[34]</sup>	Italy	≤ 2.5 2.5-4.0 4.0-6.5 6.5-28.9	OR = 1.0 OR = 0.6 (95%CI: 0.4-1.0) OR = 0.5 (95%CI: 0.3-0.7) OR = 0.6 (95%CI: 0.4-1.0) P = 0.027
Parslow <i>et al</i> <sup>[28]</sup>	United Kingdom	1.5-3.2 3.2-14.9 14.9-40.0	OR = 1.0 OR = 1.11 (95%CI: 0.98-1.26) OR = 1.27 (95%CI: 1.09-1.48)
Winkler <i>et al</i> <sup>[30]</sup>	Germany	< 9.58 ≥ 9.58	OR = 1.0 OR = 0.9 (95%CI: 0.6-1.3)
Zhao <i>et al</i> <sup>[35]</sup>	England	1-3.6 3.6-7.8 7.8-16.6	SIR = 1.11 (95%CI: 0.96-1.29) SIR = 0.99 (95%CI: 0.85-1.15) SIR = 0.90 (95%CI: 0.77-1.05) $\chi^2 = 3.8$ , P = 0.048
van Maanen <i>et al</i> <sup>[31]</sup>	The Netherland	< 10 10-25 ≥ 25 0.2-2.1 2.1-6.4 6.4-41.2	SIR = 0.99 (95%CI: 0.93-1.06) SIR = 0.99 (95%CI: 0.84-1.14) SIR = 1.45 (95%CI: 0.85-2.07) SIR = 1.02 (95%CI: 0.92-1.13) SIR = 0.95 (95%CI: 0.85-1.06) SIR = 1.02 (95%CI: 0.92-1.12)
Casu <i>et al</i> <sup>[32]</sup>	Italy	Approximately 10	Simple correlation = -0.17, P = NS
Samuelsson <i>et al</i> <sup>[33]</sup>	Sweden	0-80	OR = 1.32 (95%CI: 1.06-1.64), P = 0.013
Moltchanova <i>et al</i> <sup>[36]</sup>	Finland	0.2-6.64	Posterior mean unit effect = 0.0026 (95%CI: -0.0093-0.0138)
Kostraba <i>et al</i> <sup>[29]</sup>	United States	0-8.2	Correlation = 0.23, P = 0.07

OR: Odds ratio; SIR: Standardized incidence ratio; NS: No significance.

**Table 2** Summary of results from ecological, case-control and cohort studies of mean nitrite levels from drinking water in relation to incidence of type 1 diabetes

Ref.	Country	Exposure levels (mg/L)	Findings
Winkler <i>et al</i> <sup>[30]</sup>	Germany	< 0.009 ≥ 0.009 < 0.009 ≥ 0.009	OR for $\beta$ -cell autoimmunity = 1.0 OR for $\beta$ -cell autoimmunity = 0.6 (95%CI: 0.4-1.0), P = 0.07 OR for type 1 diabetes = 1.0 OR for type 1 diabetes = 1.5 (95%CI: 0.6-3.5), P = 0.074
Samuelsson <i>et al</i> <sup>[33]</sup>	Sweden	0.02-0.16	OR = 0.36 (95%CI: 0.06-2.03), P = 0.25

OR: Odds ratio.

drinking water) related to risk of T1DM. It should be noted that the highest intakes of dietary nitrate in this population were much lower than the ADI limit value (9 mg/d vs 259 mg/d for an adult subject) whereas dietary nitrite intakes in the highest quartile were higher than the recommended values (4.82 mg/d vs 4.2 mg/d for adults). An accurate estimation of nitrate intakes from diet and assessment of the individual's drinking water intakes may be considered as important strengths of this study.

## NITROSAMINE EXPOSURE AND THE RISK OF T1DM

N-nitrosodiethylamine and nitrosodimethylamine are two main nitrosamine compounds that contaminate food and water sources; the major known sources of dietary volatile nitrosamines are nitrite-cured meats, especially sausage and fried bacon<sup>[39,40]</sup>. Nitrosamines mediate their adverse effects due to induction of DNA damage,

oxidative stress, lipid peroxidation, and activation of inflammatory signalling pathways, which lead to increased cellular degeneration and death<sup>[41]</sup>.

For the first time in 1981, in a study of children aged 0-14 year in Island, Helgason *et al*<sup>[42]</sup> provided some primary evidence for the potential role of dietary intakes of nitrosamines in the development of T1DM. Subsequent studies have reported conflicting results. Findings of a case-control study of Australian, children aged 0-15 year, rejected this hypothesis and showed that those children who consumed higher amounts of foods containing nitrosamines did not have an increased risk of diabetes; the odds (95%CI) of T1DM were 0.71 (0.44-1.14) and 1.07 (0.66-1.74), in the middle and highest tertile compared to the lowest tertile of nitrosamine-containing foods, respectively<sup>[14]</sup>.

A prospective case-control study of Swedish children, aged 0-14 year, dietary frequency of nitrosamines rich foods including smoked fish, bacon and sausage, increased risk of T1DM in a dose-response manner<sup>[27]</sup>. Dietary exposure of nitrosamines was also positively

related to increased risk of T1DM (OR = 1.73, 95%CI: 1.23-2.44 and OR = 2.56, 95%CI: 1.83-3.59 in the medium and high categories, respectively)<sup>[27]</sup>. Further analysis stratified for different levels of dietary protein intakes, showed that higher nitrosamine intake was risk factor for diabetes, only in the presence of higher levels of protein (OR = 2.08, 95%CI: 0.94-4.60; OR = 2.12, 95%CI: 1.11-4.04,  $P = 0.03$ )<sup>[27]</sup>.

Another case-control study of Canadian children indicated no significant association between nitrosamines intakes and risk of T1DM (OR = 0.57, 95%CI: 0.21-1.57; OR = 0.66, 95%CI: 0.18-2.45; OR = 0.62, 95%CI: 0.19-2.00; in the second, third and fourth quartiles,  $P = 0.51$ ); daily intakes of nitrosamines were estimated < 0.01, 0.01-0.03, 0.03-0.04, and  $\geq 0.4$  mg/d across the quartile categories<sup>[38]</sup>.

## CONCLUSION

Ecologic surveys, case-control and cohort studies have indicated conflicting results in relation to nitrate-nitrite exposure from drinking water and the risk of T1DM. A null, sometimes even a negative association has been mainly reported in populations with a mean nitrate levels < 25 mg/L in drinking water, whereas increased risk of T1DM was reported in regions with maximum nitrate levels > 40-80 mg/L. Limited data are available regarding potential diabetogenic effects of nitrite from drinking water, a hypothesis not yet confirmed. Inconsistent findings of the studies may be attributed to a wide variation in nitrate-nitrite exposure, different cut off points used for definition of nitrate-nitrite exposure, differences in the duration of exposure and variation in potential confounding variables, adjusted in the statistical models. Lack of significant association between dietary nitrate intakes with the risk of T1DM, observed in previous studies, may be attributed to mean nitrate intakes lower than ADI. There is evidence which indicates dietary exposure of nitrite may be risk factor for development of T1DM, an effect however seems to be significant in a higher range of acceptable limits. Current data regarding dietary exposure of nitrosamine and development of T1DM is also inconsistent. To conclude findings of previous studies on nitrate-nitrite exposure and risk of T1DM, it should be noted that most studies reviewed had an ecological nature; they provided only an indirect crude estimation of exposure and described only the association between the incidence of T1DM and average level of exposure in a set of data. Considering the fact that nitrate exposure should be assessed based on individual's intake, overall estimation according to nitrate levels of water supplies, lack of data on amount of drinking water and dietary intakes of nitrate-containing foods, were main limitations of previous studies which could lead to potential misclassification of exposure; findings therefore should be considered conservatively. Relevant timing of exposure is also an important issue in assessment of the possibly diabetogenic effect of nitrate, somewhat neglected in the previous studies.

In future studies, a more accurate estimation of nitrate-nitrite exposure at an individual level is recommended to examine the potential effects on  $\beta$ -cell destruction and development of T1DM. Taking into account islet autoimmunity status and assessment of islet autoantibody levels such as insulin autoantibodies, glutamic acid decarboxylase, and IA-2, should also be considered in future investigations of the association between nitrate exposure and the risk of T1DM development, determine the role of nitrate-nitrite at different stages of the disease as initiators, promoters or trigger of the T1DM.

It should be noted the studies investigated possible association of nitrate-nitrite exposure and the risk of T1DM, are mainly limited to European countries, especially high-incidence rate populations including Sweden, Finland, England, Germany and Italy. It is also noteworthy that epidemiological investigations on diabetogenic effects of nitrate-nitrite exposure was of interest during two past decades, and scientific communities have been silent on this issue in recent years; low nitrate-nitrite exposure levels in the mentioned countries may be a reason for this trend.

Considering to an increasing trend of T1DM along with an elevated nitrate-nitrite exposure due to increased use of fertilizers and nitrite-containing food additives, additional research is critical to clarify potential harmful effects of nitrate-nitrite-nitrosamine exposure on  $\beta$ -cell autoimmunity and the risk of T1DM. Given that the incidence of T1DM is alarming among previously secured populations including Middle East, Asian and African countries, and nitrate-contaminated drinking water is currently a public health problem among these populations<sup>[43,44]</sup>, clarifying of the issue should be considered as a public health priority in developing countries.

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## REFERENCES

- 1 **Chiang JL**, Kirkman MS, Laffel LM, Peters AL. Type 1 diabetes through the life span: a position statement of the American Diabetes Association. *Diabetes Care* 2014; **37**: 2034-2054 [PMID: 24935775 DOI: 10.2337/dc14-1140]
- 2 **International Diabetes Federation**. The IDF Diabetes Atlas. 7th ed. [updated 2015 Nov 29]. Available from: URL: <http://www.diabetesatlas.org/component/attachments/?task=download&id=70>
- 3 **Diaz-Valencia PA**, Bougnères P, Valleron AJ. Global epidemiology of type 1 diabetes in young adults and adults: a systematic review. *BMC Public Health* 2015; **15**: 1-15 [DOI: 10.1186/s12889-015-1591-y]
- 4 **Diamond Project Group**. Incidence and trends of childhood Type 1 diabetes worldwide 1990-1999. *Diabet Med* 2006; **23**: 857-866 [PMID: 16911623 DOI: 10.1111/j.1464-5491.2006.01925.x]
- 5 **Kumar KM**. Incidence trends for childhood type 1 diabetes in India. *Indian J Endocrinol Metab* 2015; **19**: S34-S35 [PMID: 25941646 DOI: 10.4103/2230-8210.155378]

- 6 **El-Ziny MA**, Salem NA, El-Hawary AK, Chalaby NM, Elsharkawy AA. Epidemiology of childhood type 1 diabetes mellitus in Nile Delta, northern Egypt - a retrospective study. *J Clin Res Pediatr Endocrinol* 2014; **6**: 9-15 [PMID: 24637304 DOI: 10.4274/Jcrpe.1171]
- 7 **Patterson CC**, Dahlquist GG, Gyürüs E, Green A, Soltész G. Incidence trends for childhood type 1 diabetes in Europe during 1989–2003 and predicted new cases 2005–20: a multicentre prospective registration study. *Lancet* 2009; **373**: 2027-2033 [PMID: 19481249 DOI: 10.1016/S0140-6736(09)60568-7]
- 8 **Hussen HI**, Persson M, Moradi T. The trends and the risk of type 1 diabetes over the past 40 years: an analysis by birth cohorts and by parental migration background in Sweden. *BMJ Open* 2013; **3**: e003418 [PMID: 24176793 DOI: 10.1136/bmjopen-2013-003418]
- 9 **Mejía-León ME**, Barca AM. Diet, Microbiota and Immune System in Type 1 Diabetes Development and Evolution. *Nutrients* 2015; **7**: 9171-9184 [PMID: 26561831 DOI: 10.3390/nu7115461]
- 10 **Majeed AA**, Hassan K. Risk Factors for Type 1 Diabetes Mellitus among Children and Adolescents in Basrah. *Oman Med J* 2011; **26**: 189-195 [PMID: 22043414 DOI: 10.5001/omj.2011.46]
- 11 **Wu YL**, Ding YP, Gao J, Tanaka Y, Zhang W. Risk factors and primary prevention trials for type 1 diabetes. *Int J Biol Sci* 2013; **9**: 666-679 [PMID: 23904791 DOI: 10.7150/ijbs.6610]
- 12 **Knip M**, Veijola R, Virtanen SM, Hyöty H, Vaarala O, Akerblom HK. Environmental triggers and determinants of type 1 diabetes. *Diabetes* 2005; **54** Suppl 2: S125-S136 [PMID: 16306330 DOI: 10.2337/diabetes.54.suppl\_2.S125]
- 13 **Virtanen SM**, Knip M. Nutritional risk predictors of beta cell autoimmunity and type 1 diabetes at a young age. *Am J Clin Nutr* 2003; **78**: 1053-1067 [PMID: 14668264]
- 14 **Verge CF**, Howard NJ, Irwig I, Simpson JM, Mackerras D, Silink M. Environmental factors in childhood IDDM. A population-based, case-control study. *Diabetes Care* 1994; **17**: 1381-1389 [PMID: 7882806 DOI: 10.2337/diacare.17.12.1381]
- 15 **Bodin J**, Stene LC, Nygaard UC. Can exposure to environmental chemicals increase the risk of diabetes type 1 development? *Biomed Res Int* 2015; **2015**: 208947 [PMID: 25883945 DOI: 10.1155/2015/208947]
- 16 **Hord NG**, Tang Y, Bryan NS. Food sources of nitrates and nitrites: the physiologic context for potential health benefits. *Am J Clin Nutr* 2009; **90**: 1-10 [PMID: 19439460 DOI: 10.3945/ajcn.2008.27131]
- 17 **WTO**. Opinion of the Scientific Panel on Contaminants in the Food chain on a request from the European Commission to perform a scientific risk assessment on nitrate in vegetables, 2008
- 18 **Organization WH**. Nitrate and nitrite in drinking-water; Background document for development of WHO Guidelines for Drinking-water Quality. World Health Organization, 2011: 23
- 19 **Ghasemi M**, Delpour AR. The NMDA receptor/nitric oxide pathway: a target for the therapeutic and toxic effects of lithium. *Trends Pharmacol Sci* 2011; **32**: 420-434 [PMID: 21492946 DOI: 10.1016/j.tips.2011.03.006]
- 20 **Lundberg JO**, Carlström M, Larsen FJ, Weitzberg E. Roles of dietary inorganic nitrate in cardiovascular health and disease. *Cardiovasc Res* 2011; **89**: 525-532 [PMID: 20937740 DOI: 10.1093/cvr/cvq325]
- 21 **Machha A**, Schechter AN. Dietary nitrite and nitrate: a review of potential mechanisms of cardiovascular benefits. *Eur J Nutr* 2011; **50**: 293-303 [PMID: 21626413 DOI: 10.1007/s00394-011-0192-5]
- 22 **Bahadoran Z**, Ghasemi A, Mirmiran P, Azizi F, Hadaegh F. Beneficial effects of inorganic nitrate/nitrite in type 2 diabetes and its complications. *Nutr Metab (Lond)* 2015; **12**: 16 [PMID: 25991919 DOI: 10.1186/s12986-015-0013-6]
- 23 **Summary TC**. Nitrate and nitrite. U.S. EPA, Toxicity and Exposure Assessment for Children's Health, 2006
- 24 **Bahadoran Z**, Mirmiran P, Ghasemi A, Kabir A, Azizi F, Hadaegh F. Is dietary nitrate/nitrite exposure a risk factor for development of thyroid abnormality? A systematic review and meta-analysis. *Nitric Oxide* 2015; **47**: 65-76 [PMID: 25889269 DOI: 10.1016/j.niox.2015.04.002]
- 25 **Longnecker MP**, Daniels JL. Environmental contaminants as etiologic factors for diabetes. *Environ Health Perspect* 2001; **109** Suppl 6: 871-876 [PMID: 11744505 DOI: 10.1289/ehp.01109s6871]
- 26 **Virtanen SM**, Jaakkola L, Räsänen L, Ylönen K, Aro A, Lounamaa R, Akerblom HK, Tuomilehto J. Nitrate and nitrite intake and the risk for type 1 diabetes in Finnish children. Childhood Diabetes in Finland Study Group. *Diabet Med* 1994; **11**: 656-662 [PMID: 7955990 DOI: 10.1111/j.1464-5491.1994.tb00328.x]
- 27 **Dahlquist GG**, Blom LG, Persson LA, Sandström AI, Wall SG. Dietary factors and the risk of developing insulin dependent diabetes in childhood. *BMJ* 1990; **300**: 1302-1306 [PMID: 2369660 DOI: 10.1136/bmj.300.6735.1302]
- 28 **Parslow RC**, McKinney PA, Law GR, Staines A, Williams R, Bodansky HJ. Incidence of childhood diabetes mellitus in Yorkshire, northern England, is associated with nitrate in drinking water: an ecological analysis. *Diabetologia* 1997; **40**: 550-556 [PMID: 9165223 DOI: 10.1007/s001250050714]
- 29 **Kostraba JN**, Gay EC, Rewers M, Hamman RF. Nitrate levels in community drinking waters and risk of IDDM. An ecological analysis. *Diabetes Care* 1992; **15**: 1505-1508 [PMID: 1468277 DOI: 10.2337/diacare.15.11.1505]
- 30 **Winkler C**, Mollenhauer U, Hummel S, Bonifacio E, Ziegler AG. Exposure to environmental factors in drinking water: risk of islet autoimmunity and type 1 diabetes--the BABYDIAB study. *Horm Metab Res* 2008; **40**: 566-571 [PMID: 18500677 DOI: 10.1055/s-2008-1073165]
- 31 **van Maanen JM**, Albering HJ, de Kok TM, van Breda SG, Curfs DM, Vermeer IT, Ambergen AW, Wolffenbuttel BH, Kleinjans JC, Reeser HM. Does the risk of childhood diabetes mellitus require revision of the guideline values for nitrate in drinking water? *Environ Health Perspect* 2000; **108**: 457-461 [PMID: 10811574 DOI: 10.1289/ehp.00108457]
- 32 **Casu A**, Carlini M, Contu A, Bottazzo GF, Songini M. Type 1 diabetes in sardinia is not linked to nitrate levels in drinking water. *Diabetes Care* 2000; **23**: 1043-1044 [PMID: 10895879 DOI: 10.2337/diacare.23.7.1043]
- 33 **Samuelsson U**, Oikarinen S, Hyöty H, Ludvigsson J. Low zinc in drinking water is associated with the risk of type 1 diabetes in children. *Pediatr Diabetes* 2011; **12**: 156-164 [PMID: 20920146 DOI: 10.1111/j.1399-5448.2010.00678.x]
- 34 **Muntoni S**, Cocco P, Muntoni S, Aru G. Nitrate in community water supplies and risk of childhood type 1 diabetes in Sardinia, Italy. *Eur J Epidemiol* 2006; **21**: 245-247 [PMID: 16547840 DOI: 10.1007/s10654-006-0014-x]
- 35 **Zhao HX**, Mold MD, Stenhouse EA, Bird SC, Wright DE, Demaine AG, Millward BA. Drinking water composition and childhood-onset Type 1 diabetes mellitus in Devon and Cornwall, England. *Diabet Med* 2001; **18**: 709-717 [PMID: 11606168 DOI: 10.1046/j.1464-5491.2001.00554.x]
- 36 **Moltchanova E**, Rytönen M, Kousa A, Taskinen O, Tuomilehto J, Karvonen M. Zinc and nitrate in the ground water and the incidence of Type 1 diabetes in Finland. *Diabet Med* 2004; **21**: 256-261 [PMID: 15008836 DOI: 10.1111/j.1464-5491.2004.01125.x]
- 37 **Cherian MP**, Al-Kanani KA, Al Qahtani SS, Yesurathnam H, Mathew AA, Thomas VS, Mathew AM, Abduljabbar MA. The rising incidence of type 1 diabetes mellitus and the role of environmental factors--three decade experience in a primary care health center in Saudi Arabia. *J Pediatr Endocrinol Metab* 2010; **23**: 685-695 [PMID: 20857841 DOI: 10.1515/JPEM.2010.23.7.685]
- 38 **Benson VS**, VanLeeuwen JA, McKinney PA, Sanchez J, Dohoo IR, Somers GH. Type 1 diabetes mellitus and drinking water nitrate concentration assessed at the lot level. *Epidemiology* 2005; **16**: S49-S50 [DOI: 10.1097/00001648-200509000-00115]
- 39 **Hotchkiss JH**. Sources of N-nitrosamine contamination in foods. *Adv Exp Med Biol* 1984; **177**: 287-298 [PMID: 6388263 DOI: 10.1007/978-1-4684-4790-3\_14]
- 40 **Lijinsky W**. N-Nitroso compounds in the diet. *Mutat Res* 1999; **443**: 129-138 [PMID: 10415436 DOI: 10.1016/S1383-5742(99)00015-0]
- 41 **de la Monte SM**, Neusner A, Chu J, Lawton M. Epidemiological trends strongly suggest exposures as etiologic agents in the pathogenesis of sporadic Alzheimer's disease, diabetes mellitus, and



- non-alcoholic steatohepatitis. *J Alzheimers Dis* 2009; **17**: 519-529 [PMID: 19363256 DOI: 10.3233/JAD-2009-1070]
- 42 **Helgason T**, Jonasson MR. Evidence for a food additive as a cause of ketosis-prone diabetes. *Lancet* 1981; **2**: 716-720 [PMID: 6116858 DOI: 10.1016/S0140-6736(81)91048-5]
- 43 **Kumar L**. A study of Nitrate contamination in ground water of Delhi, India. *Lokesh* 2013; **10**: 91-94
- 44 **Gupta I**, Salunkhe A, Rohra N, Kumar R. Groundwater quality in Maharashtra, India: focus on nitrate pollution. *J Environ Sci Eng* 2011; **53**: 453-462 [PMID: 23505824]

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## Glucagon-like peptide-1 receptor agonists favorably address all components of metabolic syndrome

Sanjay Chatterjee, Samit Ghosal, Saurav Chatterjee

Sanjay Chatterjee, Apollo Gleneagles Hospitals, Kolkata 700054, India

Samit Ghosal, Nightingale Hospital, Kolkata 700071, India

Saurav Chatterjee, Cardiovascular Diseases, St. Luke's - Roosevelt Hospital Center, New York, NY 100019, United States

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**Correspondence to:** Sanjay Chatterjee, MD, Consultant Diabetologist, Apollo Gleneagles Hospital, 58 Canal Circular Road, Kolkata 700054, India. [sanjay\\_doc@yahoo.co.in](mailto:sanjay_doc@yahoo.co.in)  
 Telephone: +91-33-23203040  
 Fax: +91-33-23205218

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### Abstract

Cardiovascular death is the leading cause of mortality for patients with type 2 diabetes mellitus. The etiology

of cardiovascular disease in diabetes may be divided into hyperglycemia *per se* and factors operating through components of metabolic syndrome (MetS). Hyperglycemia causes direct injury to vascular endothelium and possibly on cardiac myocytes. MetS is a cluster of risk factors like obesity, hyperglycemia, hypertension and dyslipidemia. The incidence of this syndrome is rising globally. Glucagon-like peptide-1 receptor agonists (GLP-1RA) are a group of drugs, which address all components of this syndrome favorably. Experimental evidence suggests that they have favorable actions on myocardium as well. Several compounds belonging to GLP-1RA class are in market now and a large number awaiting their entry. Although, originally this class of drugs emerged as a treatment for type 2 diabetes mellitus, more recent data generated revealed beneficial effects on multiple metabolic parameters. We have studied literature published between 2000 and 2016 to look into effects of GLP-1RA on components of MetS. Results from recently concluded clinical trials suggest that some of the molecules in this class may have favorable effects on cardiovascular outcome.

**Key words:** Metabolic syndrome; Diabetes; Glucagon-like peptide-1 receptor agonists; Lipids; Body weight; Microalbuminuria

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**Core tip:** The incidence of metabolic syndrome (MetS) is on the rise globally. This will have a negative impact on cardiovascular outcome. Whereas most of the anti-hyperglycemic agents have neutral or negative effects on components of MetS, glucagon-like peptide-1 receptor agonists drugs favorably address all components of MetS. By doing so, they may have a cardio protective role. We have reviewed recent literature to give an updated account on the topic. Results from recently concluded clinical trials suggest that some of the molecules in this class may have favorable effects on cardiovascular

outcome.

Chatterjee S, Ghosal S, Chatterjee S. Glucagon-like peptide-1 receptor agonists favorably address all components of metabolic syndrome. *World J Diabetes* 2016; 7(18): 441-448 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v7/i18/441.htm> DOI: <http://dx.doi.org/10.4239/wjd.v7.i18.441>

## INTRODUCTION

In 1977, Haller<sup>[1]</sup> used the term "metabolic syndrome (MetS)" for association of obesity, diabetes mellitus, lipid disorder, hyperuricemia, and hepatic steatosis which increase the risk of atherosclerosis.

In 1988, Reaven<sup>[2]</sup> observed clustering of risk factors for coronary heart disease and stroke - like central obesity, hypertension, hyperglycemia and dyslipidemia, which may have a direct relationship with insulin resistance and termed the cluster, MetS X. This is also known as insulin resistance syndrome or simply, MetS.

The prevalence of diabetes and obesity is on the rise globally. In 2002, the prevalence of MetS in the United States was 34% in the adult population<sup>[3]</sup>. This will have an impact on cardiovascular mortality and morbidity.

Some authorities have suggested other components like non-alcoholic fatty liver disease (NAFLD), Micro-albuminuria, high levels of C-reactive protein (CRP) and polycystic ovary syndrome (PCOS), as parts of MetS<sup>[4]</sup>.

Recent figures in the United States estimate that there has been a reduction in the prevalence of hypertension and dyslipidemia, but an increase is noted in central obesity and hyperglycemia in the year 2010, when compared with figures of the year 1999-2000<sup>[5]</sup>.

As defined by the 2009 Joint Scientific statement, the qualifying criteria for MetS demands the presence of any three or more of the following biological thresholds: (1) waist circumference  $\geq 102$  cm (male adults) and  $\geq 88$  cm (female adults); (2) fasting plasma glucose  $\geq 5.55$  mmol/L (100 mg/dL); (3) blood pressure of 130/85 mmHg; (4) triglycerides 1.69 mmol/L ( $\geq 150$  mg/dL); and (5) high-density lipoprotein-cholesterol (HDL-C) 1.03 mmol/L ( $< 40$  mg/dL) (male adults) and 1.29 mmol/L ( $< 50$  mg/dL) (female adults). Prescription drug use was estimated for lipid-modifying agents, anti-hypertensive, and anti-hyperglycemic medications<sup>[5,6]</sup>.

The National Health and Nutrition Examination Survey (NHANES) in 1999 and 2010 (in 2-year survey waves) estimated the prevalence of MetS in adult population ( $\geq 20$  years of age). As per the results from 1999-2000 and 2009-2010: There was a reduction in the age-adjusted prevalence of MetS (based on biologic thresholds) by 2.6% (from 25.5% to 22.9%). Further perusal of the different components of MetS during this period revealed the prevalence of hypertriglyceridemia to be decreased by 9.2% (33.5% to 24.3%), as did the hypertension by 8.3% (32.3% to 24.0%). Nevertheless the prevalence of hyperglycemia increased by 7% (12.9% to 19.9%),

as did elevated waist circumference by 10.7% (45.4% to 56.1%). These trends varied considerably by sex and race/ethnicity. Changes in the prevalence of hypertension, suboptimal triglycerides, and high-density lipoprotein-cholesterol have corresponded with increases in anti-hypertensives and drugs for dyslipidemia, respectively<sup>[6]</sup>.

As regards to obesity, the prevalence is on the rise. Results from the 2011-2012 NHANES indicate that among United States adults aged 20 and over, 33.9% are overweight (BMI 25.0-29.9), 35.1% are obese (BMI 30.0-34.9), and 6.4% are extremely obese (BMI  $\geq 35.0$ ). The survey indicated wide variation of obesity in terms of age, sex and ethnicity<sup>[7]</sup>.

Native glucagon-like peptide-1 (GLP-1) is a gut hormone, produced by L-cells of distal ileum and colon in response to entry of nutrients, and has a very short half-life of about 2 min. GLP-1 is rapidly destroyed by the circulating enzyme dipeptidyl peptidase-IV (DPP-IV). GLP-1 receptors have been found in various tissues like pancreatic islet cells, the gastrointestinal tract, nervous system, cardiovascular system, kidneys and lungs.

Glucagon-like peptide-1 receptor agonists (GLP-1RA) are structurally similar to native GLP-1, but resist degradation by the enzyme DPP-IV. GLP-1RA is a new class of injectable drugs, emerged for treatment of type 2 diabetes, but has also shown beneficial effects on weight, blood pressure and lipid parameters.

Traditionally GLP-1RAs were developed as an injectable formulation, as they were rapidly degraded by the gastrointestinal enzymes when administered orally<sup>[8]</sup>. However this scenario is expected to change in the near future, with oral semaglutide preparing to hit the market. It was strongly debated, how a complex protein structure could escape the onslaught from GI juices. In the oral preparation of semaglutide, a new carrier termed Sodium N-[8-(2-hydroxybenzoyl) Amino] Caprylate (SNAC) is co-packaged to facilitate its absorption from the gut. SNAC helps in increasing the solubility of semaglutide, as well as increases its permeability across cell membrane by increasing the local (gastric mucosa) pH<sup>[9]</sup>.

We have searched PubMed, Cochrane Library, EM-BASE and Google for articles on GLP-1RA, published between 2000 and 2016, and we have found uniformly beneficial effects of GLP-1RA on cardiovascular system, obesity, hyperglycemia, hypertension and lipids. These effects of GLP-1RA have been discussed in the following paragraphs. So, this class of drug may have a favorable effect on cardiovascular mortality and morbidity.

Several GLP-1RA are already in the market and some are in the process of development. Below is a list of GLP-1RA: (1) Exenatide (Byetta) FDA approval in 2006; (2) Liraglutide (Victoza) FDA approval in 2010; (3) Exenatide Long Acting (Bydureon) FDA approval in 2012; (4) Lixisenatide (Lyxumia) EU approval in 2013; (5) Albiglutide (Tanzeum - United States; Eperzan - EU) FDA approval in 2014; (6) Dulaglutide (Trulicity) FDA approval in 2014; (7) Taspoglutide - development has been halted due to injection site skin reaction and gastrointestinal

side effects; and (8) Semaglutide - injectable and oral - undergoing clinical trials.

Fixed ratio combination of GLP-1RA and basal insulin are also available treatment option for patients with inadequate glycemic control: (1) Insulin Degludec/Liraglutide (Xultophy) EU approval in 2014; and (2) Insulin Glargine/Lixisenatide (Lixilan) Regulatory submission to USFDA in 2015.

Insulin Glargine/Lixisenatide combination has been studied in two pivotal Phase III trials; LixiLan-L and LixiLan-O, which included 1906 patients with type 2 diabetes. The results of these trials show that LixiLan significantly lowers HbA1c compared to both insulin glargine and lixisenatide. LixiLan-L included 736 patients, whose type 2 diabetes was not adequately controlled on basal insulin alone or combined to 1-2 oral antidiabetic agents. At the end of 30 wk, mean HbA1c declined from 8.1% to 6.3% with LixiLan and from 8.0% to 6.5% with glargine. With LixiLan, 84.4% achieved an HbA1c of < 7.0% compared to 78.3% with glargine. There was also 1.2 kg of weight loss with LixiLan compared to a gain of 0.4 kg with glargine. In addition, there was no difference in rates of hypoglycemia with LixiLan compared to glargine<sup>[10,11]</sup>.

## GLP-1RA - ANTIHYPERGLYCEMIC EFFECT

The antihyperglycemic effect of GLP-1RA is substantial. The action is mediated by: (1) glucose dependent insulin secretion by the pancreatic beta cells; (2) suppression of glucagon by the alpha cells; and (3) slowing of gastric emptying<sup>[12]</sup>.

In several clinical trials, with or without metformin, the GLP-RA(s) achieved HbA1c reduction between 0.9%-1.6%. There have been significant reductions of, both fasting and post-prandial plasma glucose levels (vide infra).

Treatment with exenatide 10 mg, twice daily over 30 wk to patients with type 2 diabetes, produced mean reductions in HbA1c of 0.9%-1.0%, compared to placebo, when added to metformin, a sulfonylurea or combination of both<sup>[13]</sup>.

In another 26 wk controlled trial, extended-release exenatide injection once weekly, produced a mean HbA1c reduction of 1.6%, as opposed to reduction of 0.9% by exenatide twice daily ( $P < 0.0001$ )<sup>[14]</sup>.

In a trial comparing exenatide twice daily vs liraglutide once daily, greater post-breakfast plasma glucose lowering was seen with the former while greater fasting plasma glucose was seen with the latter. There was equivalent impact on post-lunch plasma glucose excursion<sup>[15]</sup>.

In another study, adding liraglutide to failing metformin and sulfonylurea therapy, resulted in superior reduction in HbA1c (-1.33%) vs basal insulin glargine (-1.09%), and this difference was statistically significant ( $P = 0.0015$ )<sup>[16]</sup>.

In a head-to-head trial comparing glycemic efficacy

of albiglutide once weekly vs liraglutide once daily, the latter was found to be more powerful. Patients with type 2 diabetes, inadequately controlled with oral antihyperglycemic agents, were randomized to receive either albiglutide 30 mg once-weekly ( $n = 422$ ) or liraglutide uptitrated from 0.6 mg daily to 1.8 mg once daily ( $n = 419$ ). At the end of 32 wk, there was HbA1c reduction of 0.78% in the albiglutide group and 0.99% in the liraglutide group; treatment difference was 0.21%. However, gastrointestinal side effects were less in the albiglutide group and injection-site reaction was less in liraglutide group<sup>[17]</sup>.

Another head-to-head trial (AWARD-1) compared the efficacy and safety of dulaglutide against exenatide. Patients with type 2 diabetes, receiving metformin (1.5 to 3.0 g) and pioglitazone (30-45 mg) were randomized to four groups of treatment: Dulaglutide 1.5 mg weekly, dulaglutide 0.75 mg weekly, exenatide 10 µg daily, or placebo (placebo-controlled period: 26 wk). Mean baseline HbA1c was 8.1%. Change of HbA1c from baseline to the end of study was  $-1.51\% \pm 0.06\%$  for dulaglutide 1.5 mg,  $-1.30\% \pm 0.06\%$  for dulaglutide 0.75 mg,  $-0.99\% \pm 0.06\%$  for exenatide, and  $-0.46\% \pm 0.08\%$  for placebo. Dulaglutide, at both doses, was superior to placebo at 26 wk ( $P < 0.001$ ) and exenatide at 26 and 52 wk ( $P < 0.001$ ). More number of patients reached HbA1c targets with dulaglutide 1.5 mg and 0.75 mg than with placebo and exenatide (all  $P < 0.001$ ). Incidence of hypoglycemia, at 26 and 52 wk, was lower in patients receiving dulaglutide 1.5 mg than in the exenatide group; no dulaglutide-treated patients reported severe hypoglycemia. The common gastrointestinal adverse events for dulaglutide were transient, mild to moderate nausea, vomiting, and diarrhea<sup>[18]</sup>.

The first phase 3a trial results of semaglutide, a once-weekly administered GLP-1RA were announced recently in July, 2015. In this placebo controlled trial, semaglutide was administered in once-weekly doses of 0.5 mg and 1.0 mg, as monotherapy for 30 wk in 388 type 2 diabetes patients, previously on exercise and diet. The trial results showed that from a mean baseline HbA1c of 8.1%, with doses of 0.5 and 1.0 mg of semaglutide, achieved reduction in HbA1c of 1.5% and 1.6%, respectively, compared to no change in the placebo group. Seventy four percent and 73% of the people treated with 0.5 mg and 1.0 mg semaglutide, respectively, achieved the HbA1c target below 7%, compared with 25% of the people treated with placebo<sup>[19]</sup>.

The large amount of data accumulated with the use of different GLP-1RA shows a significant reduction in blood glucose values, with a greater drop seen with higher baseline values of HbA1c, in a dose-dependent manner<sup>[12]</sup>.

## GLP-1RA: EFFECTS ON SYSTOLIC AND DIASTOLIC BLOOD PRESSURE

In short term clinical trials (approximately 26 wk), on



patients with type 2 diabetes and hypertension, GLP-1RA(s) have produced a reduction of 1-7 mmHg of systolic blood pressure (SBP), whereas reduction in diastolic blood pressure (DBP) was variable.

A meta-analysis of 16 randomized controlled trials, with 3443 subjects in the GLP-1RA therapeutic arm and 2417 subjects in the control arm, studied effects on blood pressure. The GLP-1RA exenatide, reduced SBP significantly in comparison to both placebo and insulin glargine, with mean differences of -5.24 and -3.46 mmHg, respectively ( $P < 0.00001$  for both). In the exenatide-treated group, mean DBP reduction was 5.91 mmHg, compared with the placebo group, -0.99 mmHg ( $P < 0.00001$ ). The meta-analysis studied changes in systolic blood pressure. Results showed a mean reduction of 5.60 mmHg and 2.38 mmHg in the 1.2 and 1.8 mg treatment arms with liraglutide respectively, as compared to placebo and glimepiride arms ( $P < 0.00001$ ;  $P = 0.05$  respectively).

In the 1.8-mg-treated group, liraglutide significantly reduced SBP, compared to placebo and glimepiride treatment, with mean differences of -4.49 and -2.62 mmHg, respectively ( $P < 0.00001$ , and  $P < 0.00001$ , respectively)<sup>[20]</sup>.

In a study duration of 26 wk, the SBP reduction achieved with exenatide, 10 µg twice daily and liraglutide, 1.8 mg daily were found to be similar (-2.0 mmHg vs -2.5 mmHg, respectively;  $P = 0.6409$ ). An additional 14 wk follow-up of the subjects in a partial cross-over design revealed no significant difference in the SBP reduction ( $\Delta$ SBP of 3.8 mmHg, when patients were switched from exenatide to liraglutide and ( $\Delta$ SBP of 2.2 mmHg for patients, who continued on liraglutide).

It is interesting to note that blood pressure changes took place before loss of weight was observed-so the effect on blood pressure was independent of weight loss<sup>[15]</sup>.

## GLP-1RA: EFFECTS ON LIPIDS

GLP-1RAs have variable effects on different components of lipid profile, the highest being on the triglycerides. Nevertheless, beneficial effects in the LDL cholesterol and HDL cholesterol may also be noted.

Both exenatide and liraglutide resulted in a significantly greater reduction in triglycerides. After 26 wk, there was a significantly greater reduction of triglyceride with liraglutide, 1.8 mg daily as compared to exenatide (-0.22 to -0.40 mmol/L;  $P = 0.0485$ )<sup>[15]</sup>.

In a study from Greece, 20 obese type 2 diabetes patients were randomized to receive, either liraglutide or exenatide treatment and underwent a standardized meal tolerance test, early in the morning, after 10 h fast at baseline and after a two-week treatment period. Both exenatide and liraglutide, were equally effective in lowering postprandial lipemia, after the first administration and after 2 wk of treatment<sup>[21]</sup>.

In a recent prospective study, the impact of GLP-1 analogs on carotid intima media thickness (CIMT) was

assessed using lipid sub-fractions as surrogates. As MetS predisposes an individual to high cardiovascular risk, a reference to this study, may be relevant, to the topic under discussion. Adding liraglutide to type 2 diabetes patients, already on metformin and low CV risk, resulted in statistically significant ( $P < 0.001$ ) improvement in total cholesterol and triglyceride (10% drop from baseline), LDL-cholesterol (19% reduction from baseline) and increase in HDL-C (18% increment from baseline). There was a significant decrease in CIMT from baseline, however, this effect was found to be independent of changes in plasma glucose or lipids<sup>[22]</sup>.

What remains to be determined from long-term prospective trials is, whether these modest improvements in lipids will translate into cardiovascular benefit or not.

## GLP-1RA: EFFECTS ON MICROALBUMINURIA

In a study of 16 wk duration on patients with type 2 diabetes, comparing GLP-1RA exenatide with glimepiride, improvement of glucose control was similar, but a 24-h urinary albumin was reduced by 40% in exenatide group, compared to 5% reduction in glimepiride group. Apart from that, urinary transforming growth factor-beta and type IV collagen in the exenatide group were also significantly reduced, compared to, no change in glimepiride-treated group<sup>[23]</sup>.

Both exendin-4 (exenatide) and liraglutide ameliorated albuminuria, decreased oxidative stress and inflammatory cytokines, in a rat model of diabetic nephropathy. In the exendin-4 study, glomerular macrophage infiltration was prevented by suppression of ICAM-1 production on glomerular endothelial cells and by inhibition of pro-inflammatory cytokine release from macrophages<sup>[24]</sup>.

Clinical experiences with liraglutide from real-life scenario demonstrated significant improvements in urinary albumin excretion rates, as well as decline in eGFR. In an Indian data on type 2 diabetic patients, with mean duration of diabetes of approximately 12 years and baseline clinical albuminuria, there was statistically significant reduction in urinary albumin excretion rate ( $P < 0.05$ ), after 12 wk of treatment with liraglutide<sup>[25]</sup>.

## GLP-1RA: EFFECT ON BODY WEIGHT

The mechanisms linking appetite to weight gain has both peripheral sensory inputs and central response. GLP-1RA(s) have consistently demonstrated weight loss in all the clinical trials. Nausea and gastrointestinal slowing were initially postulated as the major mechanisms. However, weight loss was documented independent of gastrointestinal effects. In addition, weight loss was seen with liraglutide despite tachyphylaxis at gastric level. Hence, GLP-1RA(s) are responsible for weight loss by mechanisms, interfering both at central and peripheral sites. Recent studies using structural and functional

**Table 1** Body weight parameters: Baseline<sup>[24]</sup>

	BMI (kg/m <sup>2</sup> )	Body weight (kg)	WC (cm)
Metformin	36.6 ± 3.5	103.2 ± 6.3	122.3 ± 7.0
Liraglutide	39.3 ± 4.2	108.9 ± 15.1	124.9 ± 9.9
COMBI	37.6 ± 5.1	105.5 ± 20.6	121.9 ± 17.7

COMBI: Combination of liraglutide and metformin; WC: Waist circumference; BMI: Body mass index.

**Table 2** Post-treatment (3-mo) body weight parameters<sup>[24]</sup>

	BMI (kg/m <sup>2</sup> )	Body weight (kg)	WC (cm)
Metformin	36.1 ± 3.8	102 ± 6.8	120.7 ± 7.8
Liraglutide	37.6 ± 5.1	105.1 ± 13.8	121.7 ± 9.6
COMBI	35.5 ± 5.5	99.0 ± 21.2	116.4 ± 18.4

COMBI: Combination of liraglutide and metformin; WC: Waist circumference; BMI: Body mass index.

imaging techniques have demonstrated, reduced activity in the limbic system of the brain, as well as improved hypothalamic connectivity, leading to early satiety and modification of feeding behavior<sup>[26-28]</sup>.

A recent study, comparing the effects of metformin monotherapy vs liraglutide monotherapy vs combination (COMBI) of both, in patients with polycystic ovary syndrome, documented impressive results as far as weight loss and reduction in waist circumference were concerned. Mean weight loss with COMBI was greatest,  $6.5 \pm 2.8$  kg followed by liraglutide  $3.8 \pm 3.7$  kg and only about  $1.2 \pm 1.4$  kg with metformin. It is interesting to note that there was a significant reduction in waist circumference in the liraglutide arm ( $3.2 \pm 2.9$  cm) and in the COMBI arm ( $5.5 \pm 3.8$  cm) (Tables 1 and 2). Seventeen patients with PCOS recruited in this study had MetS (6 in metformin group, 4 in COMBI and 7 in liraglutide group). MetS persisted in all the 6 women in metformin arm at the end of the trial whereas it resolved in 3 women in both liraglutide and COMBI groups<sup>[29]</sup>.

Two different doses of liraglutide, 1.8 mg or 3 mg daily, were tried in the SCALE Diabetes trial in patients with type 2 diabetes and obesity or overweight. A total of 846 patients were randomized to receive liraglutide 3 mg daily or liraglutide 1.8 mg daily or placebo in addition to lifestyle intervention. Mean baseline weight was 105.7 kg with liraglutide (3.0-mg dose arm), 105.8 kg with liraglutide (1.8-mg dose arm), and 106.5 kg with placebo. Mean weight loss was 6.4 kg with liraglutide (3.0-mg dose), 5.0 kg with liraglutide (1.8-mg dose), and 2.2 kg with placebo<sup>[30]</sup>.

The results of SUSTAIN 1 trial are highly encouraging in terms of body weight reduction. The absolute weight reduction with 0.5 mg and 1.0 mg semaglutide are 3.8 kg and 4.6 kg as compared to 1 kg weight loss in the placebo arm respectively<sup>[19]</sup>.

## GLP-1RA AND HEPATIC AND MUSCLE INSULIN RESISTANCE

In a study, the effect of exenatide on muscle glucose uptake and hepatic glucose production (HGP) was studied in non-diabetic (control) and streptozotocin plus high fat diet induced diabetic rats. With hyperinsulinemic-euglycemic clamp, glucose uptake into gastrocnemius muscles was measured. In the diabetic rats, exenatide reduced the basal production of glucose ( $94.70 \pm 13.46$   $\mu$ mol/kg per minute vs  $121.07 \pm 16.55$   $\mu$ mol/kg per minute,  $P < 0.01$ ). This was effect of exenatide on HGP.

Also, there was increased glucose uptake into muscle ( $0.24 \pm 0.02$   $\mu$ mol/g per minute vs  $0.17 \pm 0.02$   $\mu$ mol/g per minute,  $P < 0.01$ ) - an effect on increased muscle insulin sensitivity. These effects of exenatide were absent in the non-diabetic rats<sup>[31]</sup>.

## GLP-1RA AND NON-ALCOHOLIC FATTY LIVER DISEASE

As mentioned earlier in the Introduction, some authorities have suggested NAFLD to be a component of MetS. In an interesting review article from Italy, the authors have shown strong correlation of hepatic fat deposition and MetS. They have also commented that, NAFLD is the hepatic component of MetS<sup>[32]</sup>.

In another review article, recently published, the authors concluded NAFLD as a risk factor for type 2 diabetes (28 longitudinal studies) and also for MetS (19 longitudinal studies). As regards to being a part of MetS, the issue has been complicated by documentation of high grade steatosis not associated with insulin resistance. On the contrary, a low-grade fatty liver was found to be genetic angle to this story and a direct cause and effect relationship, is not yet evident. The authors concluded that, NAFLD could be considered as a precursor to MetS, instead of a component of the same<sup>[32,33]</sup>.

Liraglutide was found to be effective in improving NAFLD and non-alcoholic steato-hepatitis (NASH). In a study conducted in Japan, the effect of liraglutide on NAFLD was compared to sitagliptin and pioglitazone. Treatment with liraglutide, significantly reduced liver enzymes, HbA1c and body weight<sup>[34]</sup>.

The effect of liraglutide on NASH, was studied in the recently published LEAN (Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis) trial. Patients were assessed with histology prior to, and at end of study (48 wk). More patients in the 1.8 mg liraglutide arm achieved histological resolution, compared to the placebo arm. However, this was a study in a very small group of patients (26 patients in each arm). Hence a study on a larger population of patients with longer duration needs to be done to come to a definitive conclusion<sup>[35]</sup>.

## GLP-1RA AND CORONARY HEART DISEASE

We have seen that, GLP-1RA have favorable actions on components of MetS. Some studies have looked into

their direct effects on coronary artery disease and left ventricular function.

In a study from South Korea, 58 patients with ST-segment elevation myocardial infarction and thrombolysis were put on either exenatide twice daily or placebo. After six months, there was significant reduction of infarct size in the exenatide group, compared to the placebo group. There was also improvement of left ventricular function in the exenatide group, in comparison to placebo<sup>[36]</sup>.

In a recently published meta-analysis of 37 clinical trials with different GLP-1RA, of duration from 24 wk to 208 wk, compared with placebo, or pioglitazone or dipeptidyl peptidase-4 inhibitors, a favorable effect on major adverse cardiovascular event (MACE) was observed with GLP-1RA(s). In placebo-controlled trials, Mantel-Haenszel odds ratio for MACE for exenatide, liraglutide and taspoglutide was 0.45 (0.20-1.02), 0.60 (0.22-1.62) and 0.50 (0.03-8.06), respectively; number of trials 6, 5 and 1 respectively;  $P = 0.055$ , 0.31 and 0.62 respectively for the three GLP-1RA(s)<sup>[37]</sup>.

The result of "Evaluation of Lixisenatide in Acute Coronary Syndrome (ELIXA)" study was slightly disappointing. The study recruited patients with type 2 diabetes who had a myocardial infarction or who had been hospitalized for unstable angina within the previous 180 d, to receive either lixisenatide or placebo (1:1 randomization), in addition to locally determined standards of care. The primary composite end points were cardiovascular death, myocardial infarction, stroke, or hospitalization for unstable angina. A total number of 6068 patients were randomized and were followed for a median of 25 mo. A primary end-point event occurred in 406 patients (13.4%) in the lixisenatide group and in 399 (13.2%) in the placebo group [hazard ratio (HR) for lixisenatide, 1.02; 95%CI: 0.89-1.17], which showed the non-inferiority of lixisenatide to placebo ( $P < 0.001$ ) but did not show superiority. There was no significant between-group difference in the rate of hospitalization for heart failure (HR in the lixisenatide group, 0.96; 95%CI: 0.75-1.23) or the rate of death (HR = 0.94; 95%CI: 0.78-1.13). Lixisenatide treatment was not associated with a higher rate of severe hypoglycemia, pancreatitis, pancreatic neoplasms, or allergic reactions, compared to placebo. The ELIXA study also showed that addition of a GLP-1RA, lixisenatide, did not increase risk of myocardial infarction or hospitalization due to heart failure, in such high-risk patients with type 2 diabetes<sup>[38]</sup>.

Another trial "Liraglutide Effect and Action in Diabetes: Evaluation of cardiovascular outcome Results (LEADER)" was started in September, 2010. The trial enrolled 9340 type 2 diabetes subjects with high risk of cardiovascular disease till April 2012. LEADER is a multicenter, international, randomized, double-blind, placebo-controlled clinical trial. The primary end point is the composite outcome of the first occurrence of cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke. The result of LEADER trial will throw light regarding the cardiovascular safety of liraglutide relative

to the current standard of usual care<sup>[39]</sup>.

On March 04, 2016, in a press release, Novo Nordisk, manufacturer of liraglutide, informed the top-line result of LEADER trial. Treatment with liraglutide has demonstrated, significant reduction of cardiovascular risk in all three components of primary endpoint. The details of the trial results will be presented in the 76<sup>th</sup> Scientific Session of the American Diabetes Association in June 2016<sup>[40]</sup>.

Once weekly injectable GLP-1RA semaglutide, that has recently completed its Phase 3a clinical trials, has also shown significant reduction in the risk of major adverse cardiovascular events, in its long-term cardiovascular safety SUSTAIN-6 trial. This was announced very recently by its manufacturer on 28 April 2016. SUSTAIN 6 is a 2-year trial to evaluate cardiovascular and other long-term outcomes with semaglutide in approximately 3300 people with type 2 diabetes<sup>[41]</sup>.

A recently published meta-analysis looked into the cardiometabolic efficacy and adverse effects of once-weekly GLP-1RAs, in adults with type 2 diabetes. The authors studied results of clinical trials with albiglutide, dulaglutide, once-weekly exenatide, and taspoglutide and looked into cardiometabolic (primary outcome, fasting plasma glucose and HbA1c) or safety outcome. Results of a total number of 34 trials were studied. All once-weekly GLP-1RAs reduced HbA1c and fasting plasma glucose. Taspoglutide 20 mg, once-weekly exenatide, and dulaglutide 1.5 mg, have also shown a reduction in body weight. The greatest difference in HbA1c reduction was found between dulaglutide 1.5 mg, and taspoglutide 10 mg (-0.4%); for fasting plasma glucose, once-weekly exenatide and albiglutide (-12.6 mg/dL), and for weight reduction, taspoglutide 20 mg, and dulaglutide 0.75 mg (-1.5 kg). Once-weekly exenatide increased heart rate compared with albiglutide and dulaglutide (1.4 to 3.2 beats/min). The risk for hypoglycemia was similar for all; use of taspoglutide 20 mg weekly was associated with the highest risk for nausea (odds ratio, 1.9 to 5.9)<sup>[42]</sup>.

## POSSIBLE MECHANISMS OF ACTION: GLP-1 RECEPTOR DEPENDENT OR INDEPENDENT

Endogenous GLP-1 can act through the GLP-1 receptors present on the endothelium, endocardium, cardiomyocytes and vascular smooth muscle cells. Once degraded by DPP-4 the intact GLP-1 (7-36) gets degraded to metabolites some of which like GLP-1 (9-36) can act independent of the GLP-1 receptor and induce vasodilation *via* the cGMP pathway<sup>[43]</sup>.

However exogenous GLP-1RA being resistant to the action of DPP-4 enzyme acts exclusively through the GLP-1 receptor and induce the metabolic and vascular effects. Amongst the various injectable GLP-1RAs, liraglutide is the only one, which is partially resistant to the degrading effect of DPP-4, due to the fatty acid side chain of the molecule, which attaches to plasma albumin

and protects the cleavage site<sup>[44]</sup>. As a result we can expect both GLP-1R dependent and independent effects on cardio-metabolic parameters from this molecule. GLP-1 (9-36) has been documented to have GLP-1R independent effects in reducing blood pressure as well as improving cardiac function post ischemia<sup>[45]</sup>. It is worth speculating whether, this additional mechanistic property was responsible for the differential CV results between ELIXA and LEADER trials.

## CONCLUSION

MetS has a strong connection with cardiovascular morbidity and mortality. Most of the conventional antihyperglycemic agents address plasma glucose excursions without having any additional impact on the other components of MetS. Some, like insulin, sulphonylureas and thiazolidinediones actually worsen certain components of MetS. The introduction of GLP-1 receptor analogs changed the picture. In addition to reducing plasma glucose, we came across, a group of drugs, which could also reduce body weight, blood pressure, lipids and improve urinary albumin excretion. The drugs have shown a trend toward favorable effects on coronary artery disease and left ventricular function. The entire composite included under the umbrella of MetS can now be tackled more effectively with one single antihyperglycemic agent. Results from recently concluded clinical trials indicate that some the drugs in this class may reduce cardiovascular risk in patients with type 2 diabetes.

## REFERENCES

- Haller H. [Epidemiology and associated risk factors of hyperlipoproteinemia]. *Z Gesamte Inn Med* 1977; **32**: 124-128 [PMID: 883354]
- Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes* 1988; **37**: 1595-1607 [PMID: 3056758 DOI: 10.2337/diabetes.37.12.1595]
- Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. *JAMA* 2002; **287**: 356-359 [PMID: 11790215 DOI: 10.1001/jama.287.3.356]
- Hanley AJ, Williams K, Festa A, Wagenknecht LE, D'Agostino RB, Haffner SM. Liver markers and development of the metabolic syndrome: the insulin resistance atherosclerosis study. *Diabetes* 2005; **54**: 3140-3147 [PMID: 16249437 DOI: 10.2337/diabetes.54.11.3140]
- Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JJ, Donato KA, Fruchart JC, James WP, Loria CM, Smith SC. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation* 2009; **120**: 1640-1645 [PMID: 19805654 DOI: 10.1161/CIRCULATIONAHA.109.192644]
- Beltrán-Sánchez H, Harhay MO, Harhay MM, McElligott S. Prevalence and trends of metabolic syndrome in the adult U.S. population, 1999-2010. *J Am Coll Cardiol* 2013; **62**: 697-703 [PMID: 23810877 DOI: 10.1016/j.jacc.2013.05.064]
- Survey Methods and Analytic Guidelines. Available from: URL: [http://www.cdc.gov/nchs/nhanes/survey\\_methods.htm](http://www.cdc.gov/nchs/nhanes/survey_methods.htm)
- Brunton S. GLP-1 receptor agonists vs. DPP-4 inhibitors for type 2 diabetes: is one approach more successful or preferable than the other? *Int J Clin Pract* 2014; **68**: 557-567 [PMID: 24499291 DOI: 10.1111/ijcp.12361]
- Emisphere's SNAC Carrier Achieves Provisional GRAS Status for Use with Nutrients Added to Foods and Dietary Supplements. [accessed 2016 Apr 26]. Available from: URL: <http://ir.emisphere.com/releasedetail.cfm?releaseid=381129>
- Venkat MV, Regier EE, Close KL. American Diabetes Association 74th Annual Scientific Sessions (ADA 2014). *J Diabetes* 2014; **6**: 491-495 [DOI: 10.1111/1753-0407.12219]
- Rosenstock J, Diamant M, Silvestre L, Souhami E, Zhou T, Fonseca V. Benefits of a fixed-ratio formulation of once-daily insulin glargine/lixisenatide (LixiLan) vs glargine in type 2 diabetes (T2DM) Inadequately Controlled on Metformin. *Diabetes* 2014; **63** Suppl 1: A1-A102 [DOI: 10.2337/db14-1-388]
- Fineman MS, Cirincione BB, Maggs D, Diamant M. GLP-1 based therapies: differential effects on fasting and postprandial glucose. *Diabetes Obes Metab* 2012; **14**: 675-688 [PMID: 22233527 DOI: 10.1111/j.1463-1326.2012.01560.x]
- DeFronzo RA, Ratner RE, Han J, Kim DD, Fineman MS, Baron AD. Effects of exenatide (exendin-4) on glycemic control and weight over 30 weeks in metformin-treated patients with type 2 diabetes. *Diabetes Care* 2005; **28**: 1092-1100 [PMID: 15855572 DOI: 10.2337/diacare.28.5.1092]
- Blevins T, Pullman J, Malloy J, Yan P, Taylor K, Schulteis C, Trautmann M, Porter L. DURATION-5: exenatide once weekly resulted in greater improvements in glycemic control compared with exenatide twice daily in patients with type 2 diabetes. *J Clin Endocrinol Metab* 2011; **96**: 1301-1310 [PMID: 21307137 DOI: 10.1210/jc.2010-2081]
- Buse JB, Rosenstock J, Sesti G, Schmidt WE, Montanya E, Brett JH, Zychma M, Blonde L. Liraglutide once a day versus exenatide twice a day for type 2 diabetes: a 26-week randomised, parallel-group, multinational, open-label trial (LEAD-6). *Lancet* 2009; **374**: 39-47 [PMID: 19515413 DOI: 10.1016/S0140-6736(09)60659-0]
- Russell-Jones D, Vaag A, Schmitz O, Sethi BK, Lalic N, Antic S, Zdravkovic M, Ravn GM, Simó R. Liraglutide vs insulin glargine and placebo in combination with metformin and sulfonylurea therapy in type 2 diabetes mellitus (LEAD-5 met+SU): a randomised controlled trial. *Diabetologia* 2009; **52**: 2046-2055 [PMID: 19688338 DOI: 10.1007/s00125-009-1472-y]
- Pratley RE, Nauck MA, Barnett AH, Feinglos MN, Ovalle F, Harman-Boehm I, Ye J, Scott R, Johnson S, Stewart M, Rosenstock J. Once-weekly albiglutide versus once-daily liraglutide in patients with type 2 diabetes inadequately controlled on oral drugs (HARMONY 7): a randomised, open-label, multicentre, non-inferiority phase 3 study. *Lancet Diabetes Endocrinol* 2014; **2**: 289-297 [PMID: 24703047 DOI: 10.1016/S2213-8587(13)70214-6]
- Wysham C, Blevins T, Arakaki R, Colon G, Garcia P, Atisio C, Kuhstoss D, Lakshmanan M. Efficacy and safety of dulaglutide added onto pioglitazone and metformin versus exenatide in type 2 diabetes in a randomized controlled trial (AWARD-1). *Diabetes Care* 2014; **37**: 2159-2167 [PMID: 24879836 DOI: 10.2337/dc13-2760]
- Novo Nordisk successfully completes first phase 3a trial with semaglutide in people with type 2 diabetes. [accessed 2016 Feb 5]. Available from: URL: <https://www.novonordisk.com/bin/get-PDF.1934243.pdf>
- Wang B, Zhong J, Lin H, Zhao Z, Yan Z, He H, Ni Y, Liu D, Zhu Z. Blood pressure-lowering effects of GLP-1 receptor agonists exenatide and liraglutide: a meta-analysis of clinical trials. *Diabetes Obes Metab* 2013; **15**: 737-749 [PMID: 23433305 DOI: 10.1111/dom.12085]
- Voukali M, Kastrinelli I, Stragalinou S, Tasiopoulou D, Paraskevopoulou P, Katsilambros N, Kokkinos A, Tentolouris N, Ioannidis I. Study of postprandial lipaemia in type 2 diabetes mellitus: exenatide versus liraglutide. *J Diabetes Res* 2014; **2014**: 304032 [PMID: 25165723 DOI: 10.1155/2014/304032]
- Rizzo M, Chandalia M, Patti AM, Di Bartolo V, Rizvi AA,



- Montalto G, Abate N. Liraglutide decreases carotid intima-media thickness in patients with type 2 diabetes: 8-month prospective pilot study. *Cardiovasc Diabetol* 2014; **13**: 49 [PMID: 24559258 DOI: 10.1186/1475-2840-13-49]
- 23 **Zhang H**, Zhang X, Hu C, Lu W. Exenatide reduces urinary transforming growth factor- $\beta$ 1 and type IV collagen excretion in patients with type 2 diabetes and microalbuminuria. *Kidney Blood Press Res* 2012; **35**: 483-488 [PMID: 22687869 DOI: 10.1159/000337929]
- 24 **Kodera R**, Shikata K, Kataoka HU, Takatsuka T, Miyamoto S, Sasaki M, Kajitani N, Nishishita S, Sarai K, Hirota D, Sato C, Ogawa D, Makino H. Glucagon-like peptide-1 receptor agonist ameliorates renal injury through its anti-inflammatory action without lowering blood glucose level in a rat model of type 1 diabetes. *Diabetologia* 2011; **54**: 965-978 [PMID: 21253697 DOI: 10.1007/s00125-010-2028-x]
- 25 **Ghosal S**. Using liraglutide in combination with insulin therapy for type 2 diabetic patients: An early clinical experience data. *Diabetes* 2011; **60** Suppl 1: A235-A352 [DOI: 10.2337/db11-868-1281]
- 26 **Meier JJ**. GLP-1 receptor agonists for individualized treatment of type 2 diabetes mellitus. *Nat Rev Endocrinol* 2012; **8**: 728-742 [PMID: 22945360 DOI: 10.1038/nrendo.2012.140]
- 27 **Min DK**, Tuor UI, Koopmans HS, Chelikani PK. Changes in differential functional magnetic resonance signals in the rodent brain elicited by mixed-nutrient or protein-enriched meals. *Gastroenterology* 2011; **141**: 1832-1841 [PMID: 21802388 DOI: 10.1053/j.gastro.2011.07.034]
- 28 **Schlögl H**, Kabisch S, Horstmann A, Lohmann G, Müller K, Lepsien J, Busse-Voigt F, Kratzsch J, Pleger B, Villringer A, Stumvoll M. Exenatide-induced reduction in energy intake is associated with increase in hypothalamic connectivity. *Diabetes Care* 2013; **36**: 1933-1940 [PMID: 23462665 DOI: 10.2337/dc12-1925]
- 29 **Jensterle Sever M**, Kocjan T, Pfeifer M, Kravos NA, Janez A. Short-term combined treatment with liraglutide and metformin leads to significant weight loss in obese women with polycystic ovary syndrome and previous poor response to metformin. *Eur J Endocrinol* 2014; **170**: 451-459 [PMID: 24362411 DOI: 10.1530/EJE-13-0797]
- 30 **Davies MJ**, Bergenstal R, Bode B, Kushner RF, Lewin A, Skjøth TV, Andreasen AH, Jensen CB, DeFronzo RA. Efficacy of Liraglutide for Weight Loss Among Patients With Type 2 Diabetes: The SCALE Diabetes Randomized Clinical Trial. *JAMA* 2015; **314**: 687-699 [PMID: 26284720 DOI: 10.1001/jama.2015.9676]
- 31 **Wu H**, Sui C, Xu H, Xia F, Zhai H, Zhang H, Weng P, Han B, Du S, Lu Y. The GLP-1 analogue exenatide improves hepatic and muscle insulin sensitivity in diabetic rats: tracer studies in the basal state and during hyperinsulinemic-euglycemic clamp. *J Diabetes Res* 2014; **2014**: 524517 [PMID: 25580440 DOI: 10.1155/2014/524517]
- 32 **Tarantino G**, Finelli C. What about non-alcoholic fatty liver disease as a new criterion to define metabolic syndrome? *World J Gastroenterol* 2013; **19**: 3375-3384 [PMID: 23801829 DOI: 10.3748/wjg.v19.i22.3375]
- 33 **Lonardo A**, Ballestri S, Marchesini G, Angulo P, Loria P. Nonalcoholic fatty liver disease: a precursor of the metabolic syndrome. *Dig Liver Dis* 2015; **47**: 181-190 [PMID: 25739820 DOI: 10.1016/j.dld.2014.09.020]
- 34 **Ohki T**, Isogawa A, Iwamoto M, Ohsugi M, Yoshida H, Toda N, Tagawa K, Omata M, Koike K. The effectiveness of liraglutide in nonalcoholic fatty liver disease patients with type 2 diabetes mellitus compared to sitagliptin and pioglitazone. *ScientificWorldJournal* 2012; **2012**: 496453 [PMID: 22927782 DOI: 10.1100/2012/496453]
- 35 **Armstrong MJ**, Gaunt P, Aithal GP, Barton D, Hull D, Parker R, Hazlehurst JM, Guo K, Abouda G, Aldersley MA, Stocken D, Gough SC, Tomlinson JW, Brown RM, Hübscher SG, Newsome PN. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. *Lancet* 2016; **387**: 679-690 [PMID: 26608256 DOI: 10.1016/S0140-6736(15)00803-X]
- 36 **Woo JS**, Kim W, Ha SJ, Kim JB, Kim SJ, Kim WS, Seon HJ, Kim KS. Cardioprotective effects of exenatide in patients with ST-segment-elevation myocardial infarction undergoing primary percutaneous coronary intervention: results of exenatide myocardial protection in revascularization study. *Arterioscler Thromb Vasc Biol* 2013; **33**: 2252-2260 [PMID: 23868944 DOI: 10.1161/ATVBAHA.113.301586]
- 37 **Monami M**, Dicembrini I, Nardini C, Fiordelli I, Mannucci E. Effects of glucagon-like peptide-1 receptor agonists on cardiovascular risk: a meta-analysis of randomized clinical trials. *Diabetes Obes Metab* 2014; **16**: 38-47 [PMID: 23829656 DOI: 10.1111/dom.12175]
- 38 **Sjöholm A**. Impact of glucagon-like peptide-1 on endothelial function. *Diabetes Obes Metab* 2009; **11** Suppl 3: 19-25 [PMID: 19878258 DOI: 10.1111/j.1463-1326.2009.01074.x]
- 39 **Deacon CF**. Potential of liraglutide in the treatment of patients with type 2 diabetes. *Vasc Health Risk Manag* 2009; **5**: 199-211 [PMID: 19436648 DOI: 10.2147/VHRM.S4039]
- 40 **Victoza®** Significantly Reduced the Risk of Major Adverse Cardiovascular Events in the LEADER Trial. [accessed 2016 May 11]. Available from: URL: <http://www.prnewswire.com/news-releases/victoza-significantly-reduced-the-risk-of-major-adverse-cardiovascular-events-in-the-leader-trial-300231181.html>
- 41 **Semaglutide** significantly reduces the risk of major adverse cardiovascular events in the SUSTAIN 6 trial. [accessed 2016 May 9]. Available from: URL: <https://globenewswire.com/news-release/2016/04/28/834013/0/en/Semaglutide-significantly-reduces-the-risk-of-major-adverse-cardiovascular-events-in-the-SUSTAIN-6-trial.html>
- 42 **Noyan-Ashraf MH**, Momen MA, Ban K, Sadi AM, Zhou YQ, Riazi AM, Baggio LL, Henkelman RM, Husain M, Drucker DJ. GLP-1R agonist liraglutide activates cytoprotective pathways and improves outcomes after experimental myocardial infarction in mice. *Diabetes* 2009; **58**: 975-983 [PMID: 19151200 DOI: 10.2337/db08-1193]
- 43 **Pfeffer MA**, Claggett B, Diaz R, Dickstein K, Gerstein HC, Køber LV, Lawson FC, Ping L, Wei X, Lewis EF, Maggioni AP, McMurray JJ, Probstfield JL, Riddle MC, Solomon SD, Tardif JC. Lixisenatide in Patients with Type 2 Diabetes and Acute Coronary Syndrome. *N Engl J Med* 2015; **373**: 2247-2257 [PMID: 26630143 DOI: 10.1056/NEJMoa1509225]
- 44 **Marso SP**, Poulter NR, Nissen SE, Nauck MA, Zinman B, Daniels GH, Pocock S, Steinberg WM, Bergenstal RM, Mann JF, Ravn LS, Frandsen KB, Moses AC, Buse JB. Design of the liraglutide effect and action in diabetes: evaluation of cardiovascular outcome results (LEADER) trial. *Am Heart J* 2013; **166**: 823-830.e5 [PMID: 24176437 DOI: 10.1016/j.ahj.2013.07.012]
- 45 **Zaccardi F**, Htike ZZ, Webb DR, Khunti K, Davies MJ. Benefits and Harms of Once-Weekly Glucagon-like Peptide-1 Receptor Agonist Treatments: A Systematic Review and Network Meta-analysis. *Ann Intern Med* 2016; **164**: 102-113 [PMID: 26642233 DOI: 10.7326/M15-1432]

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Observational Study

# Impact of diabetes mellitus on risk of cardiovascular disease and all-cause mortality: Evidence on health outcomes and antidiabetic treatment in United States adults

Longjian Liu, Barbara Simon, Jinggaofu Shi, Arshpreet Kaur Mallhi, Howard J Eisen

Longjian Liu, Jinggaofu Shi, Arshpreet Kaur Mallhi, Department of Epidemiology and Biostatistics, Drexel University Dornsife School of Public Health, Philadelphia, PA 19104, United States

Barbara Simon, Division of Endocrinology, Drexel University College of Medicine, Philadelphia, PA 19102, United States

Howard J Eisen, Division of Cardiology, Drexel University College of Medicine, Philadelphia, PA 19102, United States

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**Informed consent statement:** The current report used data from the National Center for Health Statistics (NCHS) of the United States Centers for Disease Control and Prevention CDC). All informed consents were conducted by the NCHS while the study was carried. No further informed consent forms were requested by using the dataset (<https://www.cdc.gov/nchs/nhis/>).

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**Data sharing statement:** Data used in the present study were public use data files provided by the United States CDC NCHS. Those who are interested may apply for and download from NCHS website.

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**Correspondence to:** Longjian Liu, MD, PhD, MSc, FAHA, Department of Epidemiology and Biostatistics, Drexel University Dornsife School of Public Health, 3215 Market ST, Philadelphia, PA 19104, United States. [longjian.liu@drexel.edu](mailto:longjian.liu@drexel.edu)  
 Telephone: +1-267-3596049  
 Fax: +1-267-3596201

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## Abstract

### AIM

To examine the epidemic of diabetes mellitus (DM) and its impact on mortality from all-cause and cardiovascular disease (CVD), and to test the effect of antidiabetic therapy on the mortality in United States adults.

### METHODS

The analysis included a randomized population sample of 272149 subjects ages  $\geq 18$  years who participated in the National Health Interview Surveys (NHIS) in 2000-2009. Chronic conditions (hypertension, DM and CVD) were classified by participants' self-reports of physician diagnosis. NHIS-Mortality Linked Files, and

NHIS-Medical Expenditure Panel Survey Linkage Files on prescribed medicines for patients with DM were used to test the research questions.  $\chi^2$ , Poisson and Cox's regression models were applied in data analysis.

## RESULTS

Of all participants, 22305 (8.2%) had DM. The prevalence of DM significantly increased from 2000 to 2009 in all age groups ( $P < 0.001$ ). Within an average 7.39 (SD = 3) years of follow-up, male DM patients had 1.56 times higher risk of death from all-cause (HR = 1.56, 95%CI: 1.49-1.64), 1.72 times higher from heart disease [1.72 (1.53-1.93)], 1.48 times higher from cerebrovascular disease [1.48 (1.18-1.85)], and 1.67 times higher from CVD [1.67 (1.51-1.86)] than subjects without DM, respectively. Similar results were observed in females. In males, 10% of DM patients did not use any antidiabetic medications, 38.1% used antidiabetic monotherapy, and 51.9% used  $\geq 2$  antidiabetic medications. These corresponding values were 10.3%, 40.4% and 49.4% in females. A significant protective effect of metformin monotherapy or combination therapy (except for insulin) on all-cause mortality and a protective but non-significant effect on CVD mortality were observed.

## CONCLUSION

This is the first study using data from multiple linkage files to confirm a significant increased prevalence of DM in the last decade in the United States. Patients with DM have significantly higher risk of death from all-cause and CVD than those without DM. Antidiabetic medications, specifically for metformin use, show a protective effect against all-cause and CVD mortalities.

**Key words:** Epidemic of diabetes mellitus; Cardiovascular disease; Pharmacoepidemiologic profiles; United States

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**Core tip:** The study is one of the first projects to use a 10-years nationally linked dataset. The results highlight a new epidemic of diabetes in the United States. It addresses the impact of diabetes on cardiovascular disease and all-cause mortality. The study is also one of the first studies to explore the association between glucose lowering drug use and health outcomes using health survey data from the real-world.

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## INTRODUCTION

Diabetes mellitus (DM) is the seventh leading cause

of death in the United States. Of 2543279 death certificates from all-causes in 2010, 2.9% of deaths ( $n = 73932$ ) clinically listed DM as the main cause of death, and more than 9% of deaths ( $n = 234051$ ) were attributable to DM as a comorbid cause of death in the United States. It is estimated that more than 1.4 million Americans are diagnosed with DM every year. In 2012, 29.1 million Americans, or 9.3% of the population, had DM. Of the 29.1 million, 21 million were diagnosed, and 8.1 million were undiagnosed<sup>[1]</sup>. A similar increased prevalence of DM has been estimated worldwide<sup>[2]</sup>. It is clear that DM has posed a serious public health problem in the United States and in the world<sup>[1-4]</sup>, not only because DM is a leading cause of death, but also DM is a significant risk factor for cardiovascular disease (CVD). CVD is the number one killer of the Americans<sup>[5-10]</sup>. Although the overall trend in the prevalence of DM and its impact on risk of CVD have been examined by several studies, some were limited to their small sample sizes<sup>[11]</sup>, some were limited to their study designs [such as findings from the Behavior Risk Factor Surveillance Systems that are conducted using a telephone survey with a very low response rate ( $< 40\%$ )]<sup>[12]</sup>, and some were limited to a cross-sectional analysis design<sup>[7,13]</sup>. Furthermore, patterns of antidiabetic treatment and its impact on long-term health outcomes are less known. In the present study, we aimed to examine the trend of DM, and the impact of DM on CVD (hypertension, coronary heart disease and stroke), and risk of mortality from CVD and all-cause using a nationally representative sample in the United States. Findings from the study may add new evidence of the burdens of DM to the body of the literatures, the patterns of antidiabetic medications usage, and the magnitudes of DM and drug use on all-cause and CVD mortalities.

## MATERIALS AND METHODS

Participants ages 18 years and older in the 2000-2009 National Health Interview Surveys (NHIS) were included in the study. The NHIS has been conducted annually since 1960 by the United States National Center for Health Statistics (NCHS), which is a part of the Centers for Disease Control and Prevention (CDC)<sup>[14]</sup>. NHIS is a cross-sectional household interview survey that serves as the principal source of information on the health of the noninstitutionalized civilian population of the United States<sup>[15]</sup>. Uniform sampling and interviewing processes for core variables are continuous throughout each year's survey. The sampling plan follows a multistage area probability design that permits the representative sampling of households and non-institutionalized groups. In NHIS, one adult per household is randomly chosen to participate in a completed interview from approximately 30000 households containing about 85500 persons, of them about 30000 adults ages 18 and older. Participants' vital status (alive or deceased) are followed yearly and linked to death certificates in the National Death Index system (NHIS-Mortality

Linked File). This Linked File provides an important opportunity for health professionals to estimate the risk of mortality prospectively on the basis of the NHIS participants' baseline characteristics<sup>[14]</sup>. In the present study, we applied the most recently released NHIS-Mortality Linked File, which had follow-up information for subjects who participated in NHIS in and before 2009, and followed up through the end of 2011 (December 31, 2011). We examined the past one decade trend of DM between 2000 and 2009, and risk of mortality in patients with DM. Of total 287530 participants ages 18 and older, we excluded 15381 who had missing information on prevalent DM status at baseline ( $n = 237$ ), and those who were lost to follow-up ( $n = 15144$ ) during the course of follow-up between 2000 and 2011, yielding a final analytic sample of 272149 adults ( $94.7\% = 272149/287530$ ). To examine the patterns of medication use in patients with DM, we further linked the study sample at individual participant's level with the Medical Expenditure Panel Surveys (MEPS)<sup>[16]</sup>. The NHIS, NHIS-Mortality Linked File and MEPS have been approved by the Institutional Review Board of the United States CDC NCHS and are available through the NCHS<sup>[14]</sup>. The present analysis has been approved by Drexel University Institutional Review Board (# 1605004544).

Two groups of health outcomes in patients with and without DM were examined: (1) all-cause mortality; and (2) CVD mortality. Mortality data were defined using ICD-10: Heart disease (ICD10: I00-I09, I11, I13, I20-I51), cerebrovascular disease (ICD10: I60-I69). CVD includes the two major forms of heart disease and cerebrovascular disease (ICD10: I00-I09, I11, I13, I20-I51 and ICD10: I60-I69). Predictors and covariates included: (1) demographic factors: Age, gender, race/ethnicity and education attainments (< high-school graduate, high-school graduate, and  $\geq$  college); (2) lifestyle related factors: Body mass index [BMI, calculated by weight (kg)/height ( $m^2$ )], cigarette smoking (never smoked, formerly smoked, or currently smoker), alcohol consumption (not a drinker: < 12 drinks in entire life, former drinker: No drinks in previous year, and current drinker), and physical activity. BMI was classified into four groups on the basis of the World Health Organization (WHO) definition (underweight: < 18.5, normal weight: 18.5-24.9, overweight: 25-29.9, and obese:  $\geq 30$  kg/ $m^2$ ). Physical activity status was grouped on the basis of current guidelines (active:  $\geq 150$  min per week of moderate-intensity equivalent leisure-time aerobic activity; insufficiently active: 10-149 min per week of moderate-intensity equivalent leisure-time aerobic activity); (3) CVD related chronic conditions: Hypertension, coronary heart disease (CHD) and stroke. Baseline CVD includes patients who had CHD and/or stroke. The baseline chronic conditions were classified by participants' self-reports of diagnoses made by a doctor or health professional; and (4) DM, oral glucose-lowering medication and insulin use were classified according to DM patients' prescription records.

## Statistical analysis

A serial analysis was conducted to test the study hypotheses and fit the time-events prediction models. The first group analysis included the basic characteristics description of the study participants and tested gender differences using univariate analysis, including  $t$  test for continuous variables, and  $\chi^2$  tests for categorical variables. Changes in the prevalence of DM from 2000 to 2009 by sex and ages (18-54, 55-64, 65-74 and  $\geq 75$ ) were tested using simple linear regression models. The second group analysis involved estimates of mortality rates (per 1000 person-year) from all-cause, heart disease, cerebrovascular disease, and total CVD. We used Poisson regression to calculate mortality per person years. The third group analysis estimated the hazard ratios of DM (yes/no) for the risk of mortality from all-cause, heart disease, cerebrovascular disease, and total CVD using Cox's proportional hazard regression models. In the analysis, five multivariate adjusted Cox's models were performed by gender. Model 1 adjusted for age (years) and race/ethnicity (NH-White, NH-Black, Hispanics, and other groups). Model 2 adjusted for age, race/ethnicity and education level (< higher school, high school, and  $\geq$  college). Model 3 adjusted for the covariates used in Model 2 plus three behavioral factors (smoking, alcohol consumption and physical activity). Model 4 adjusted for the covariates used in Model 3 plus hypertension. Because patients with CVD at baseline may have an increased risk of mortality, we excluded those patients in Model 5 and adjusted the same covariates as used in Model 4. Interactions of gender and DM on risk of mortality were tested using SAS Proc GENMOD. The fourth group analysis involved in estimates of the prevalence of glucose lowering medication and insulin use. We examined hazard ratios of monotherapy and combinations of glucose lowering medication and insulin use for the risk of mortalities compared to those without antidiabetic medication use. In the last group analysis, we compared baseline differences in 5 preventable factors' age-race-adjusted standardized rates (education level, as a marker for economic status, smoking, physical activity, BMI and hypertension) between males and females using logistic regression in order to explain a potential gender difference in the relative risk of all-cause and CVD mortality in patients with DM.

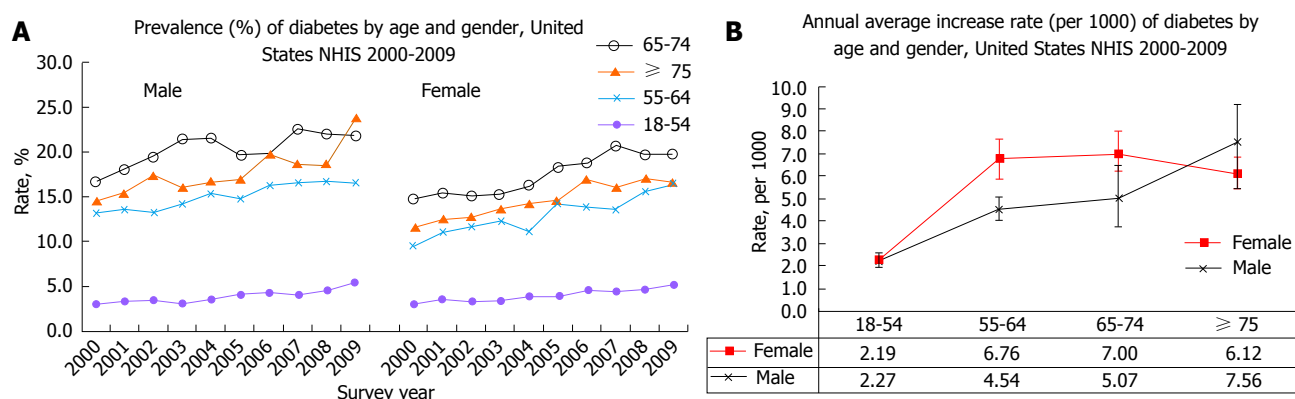
All data analyses were performed using SAS version 9.3, with complex sample modules that take the sample design of NHIS, including stratification, clustering and weight into consideration (SAS Institute, Cary, NC). Statistical significance was determined for a two-sided test at a  $P$  value < 0.05.

## RESULTS

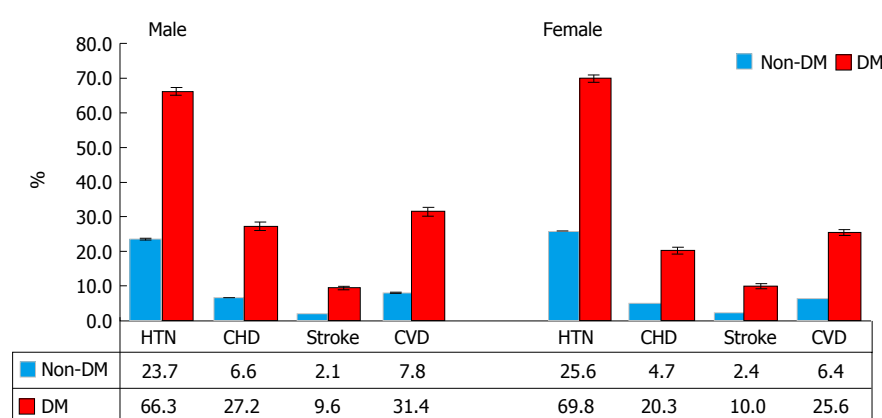
### Baseline characteristics of the study participants

Of 272149 subjects participated in 2000-2009 NHIS, 22305 (8.2%) had diabetes (male: 9892, and female: 12413). The prevalence of DM significantly increased





**Figure 1 The Burden of diabetes in the United States.** A: Prevalence (%) of diabetes by age and gender, United States NHIS 2000-2009; B: Annual average increase rate (per 1000) of diabetes by age and gender, United States NHIS 2000-2009. NHIS: National Health Interview Surveys.



**Figure 2 Prevalence (%) of comorbid hypertension, coronary heart disease, stroke and cardiovascular disease in patients with and without diabetes mellitus, United States National Health Interview Surveys 2000-2009.** HTN: Hypertension; CHD: Coronary heart disease; CVD: Cardiovascular disease; DM: Diabetes mellitus; NHIS: National Health Interview Surveys.

from 2000 to 2009 in all age groups for males and females ( $P < 0.001$ ) (Figure 1A). The annual increase rates (per 1000) were 2.27, 4.54, 5.07, and 7.56 for male aged 18-54, 55-64, 65-74 and  $\geq 75$ , respectively (test for linear trend, all  $P < 0.01$ ). The corresponding values in females were 2.19, 6.76, 7.00 and 6.12, respectively ( $P < 0.01$ ). Females had 1.49 times higher annual increase in those aged 55-64, and 1.38 higher in those aged 65-74 compared to males (Figure 1B). There were significant differences in demographic, behavior factors and medical conditions between those with and without DM in males and females (Table 1 and Figure 2).

#### Mortality from all-cause and CVD in patients with DM vs those without DM

Table 2 shows that mortality from all-cause, heart disease, cerebrovascular disease (CBVD) and CVD increased with age in subjects with or without DM. However, patients with DM had significantly higher mortality than those without DM in both males and females, except for CBVD in males aged 18-54.9 ( $P = 0.063$ ), and aged  $\geq 75$  ( $P = 0.694$ ), and in females aged  $\geq 75$  ( $P = 0.371$ ). Figure 3A depicts an overall

increase in all-cause mortality with increased age in males and females, but a greater increased trend for those aged  $\geq 65$ . Similar trend for CVD mortality is shown in Figure 3B. Subjects without DM had a much lower mortality rate from all-cause and CVD before the age of 65 as compared to those with DM and age of 65 and older.

#### Multivariate adjusted hazard ratios of DM for risk of mortality

Of the total study sample, within an average 7.39 (SD = 3) years of follow-up, the results show that after adjustment for age and race/ethnicity, male patients with DM vs non-DM had 1.56 times higher risk of death from all-cause (HR = 1.56, 95%CI: 1.49-1.64), 1.72 times higher from heart disease (HR = 1.72, 95%CI: 1.53-1.93), 1.48 times higher from CBVD (HR = 1.48, 95%CI: 1.18-1.85), and 1.67 times higher from CVD (HR = 1.67, 95%CI: 1.51-1.86), respectively (Model 1, Table 3). Similar results were observed in females. After further adjustment for the inclusion of education (Model 2), and behavior risk factors (cigarette smoking, alcohol consumption, and physical inactivity, Model 3), the corresponding HRs of DM for the risk of the mortalities

**Table 1** Baseline characteristics of participants, United States National Health Interview Surveys 2000-2009

	Male					Female				
	Non-DM ( <i>n</i> = 109507)		DM ( <i>n</i> = 9892)		<i>P</i> value	Non-DM ( <i>n</i> = 140337)		DM ( <i>n</i> = 12413)		<i>P</i> value <sup>c</sup>
	No. <sup>a</sup>	Rate <sup>b</sup> (SEP)	No. <sup>a</sup>	Rate <sup>b</sup> (SEP)		No. <sup>a</sup>	Rate <sup>b</sup> (SEP)	No. <sup>a</sup>	Rate <sup>b</sup> (SEP)	
Age, mean, yr	44.9	(0.10)	60.3	(0.14)	< 0.0001	46.6	(0.1)	60.7	(0.16)	< 0.0001
Race/ethnicity										
NH-White	71683	74.5 (0.25)	6062	70.9 (0.55)	< 0.0001	89088	73.8 (0.25)	6782	66.6 (0.55)	< 0.0001
NH-Black	13345	10.4 (0.18)	1712	14.2 (0.40)		21517	12.5 (0.20)	2923	19.2 (0.46)	
Hispanics	19251	10.7 (0.17)	1646	10.4 (0.36)		23779	9.8 (0.15)	2260	10.7 (0.36)	
Others	5228	4.4 (0.10)	472	4.5 (0.25)		5953	3.9 (0.08)	448	3.4 (0.21)	
Education										
Less than HS	19941	15.3 (0.18)	2682	24.0 (0.52)	< 0.0001	25377	15.0 (0.16)	4066	28.8 (0.50)	< 0.0001
HS Graduated	30474	28.0 (0.21)	2887	30.6 (0.58)		39612	28.4 (0.19)	3873	33.1 (0.54)	
≥ College	58314	56.6 (0.30)	4231	45.3 (0.58)		74451	56.5 (0.27)	4370	38.1 (0.53)	
Smoking status										
No smoker	54430	49.6 (0.24)	3667	36.7 (0.58)	< 0.0001	87384	60.8 (0.21)	7344	58.2 (0.51)	< 0.0001
Former smoker	26644	24.9 (0.19)	4293	44.6 (0.60)		24800	19.1 (0.14)	3075	26.4 (0.47)	
Current smoker	27507	25.5 (0.19)	1834	18.7 (0.46)		27211	20.1 (0.16)	1893	15.4 (0.35)	
Alcohol consumption										
Never	16415	14.3 (0.19)	1660	16.5 (0.43)	< 0.0001	41116	27.0 (0.23)	5159	39.7 (0.55)	< 0.0001
Former	15028	13.8 (0.15)	3348	34.0 (0.61)		19335	14.1 (0.13)	3389	28.2 (0.51)	
Current	75204	71.8 (0.22)	4668	49.5 (0.62)		77255	58.9 (0.25)	3670	32.1 (0.54)	
Exercise										
Inactive	38394	34.5 (0.32)	4485	47.0 (0.62)	< 0.0001	55965	38.9 (0.29)	6416	55.2 (0.62)	< 0.0001
Insufficiently active	27605	27.2 (0.18)	2178	24.6 (0.50)		40073	31.0 (0.18)	2724	25.1 (0.49)	
Sufficiently active	38609	38.3 (0.26)	2473	28.3 (0.59)		38637	30.0 (0.22)	2158	19.8 (0.45)	
BMI, kg/m <sup>2</sup>										
Overweight	46884	43.5 (0.18)	3595	36.2 (0.55)	< 0.0001	38254	28.1 (0.15)	3250	28.0 (0.45)	< 0.0001
Obesity	24128	22.4 (0.17)	4372	46.1 (0.59)		31193	22.4 (0.15)	6168	52.8 (0.53)	
Medical condition										
Hypertension	25582	23.7 (0.19)	6501	66.3 (0.56)	< 0.0001	35594	25.6 (0.17)	8662	69.8 (0.48)	< 0.0001
Coronary heart Dis	7020	6.6 (0.09)	2598	27.2 (0.48)	< 0.0001	6435	4.7 (0.07)	2450	20.3 (0.41)	< 0.0001
Stroke	2243	2.1 (0.05)	956	9.6 (0.30)	< 0.0001	3244	2.4 (0.05)	1233	10.0 (0.32)	< 0.0001
CVD	8434	7.8 (0.10)	3019	31.4 (0.51)	< 0.0001	8699	6.4 (0.09)	3109	25.6 (0.43)	< 0.0001

<sup>a</sup>No. = Observed number; <sup>b</sup>Rate for weighted rate using SAS for complex survey; <sup>c</sup>T test for continuous variable, and  $\chi^2$  test for categorical variables. Education ≥ College including those with associate degrees. Overweight: Body mass index (BMI) 25 to 29.9 kg/m<sup>2</sup>. Obesity: BMI ≥ 30 kg/m<sup>2</sup>. CVD: Cardiovascular disease; DM: Diabetes mellitus; SEP: Standard error of proportion.

remained statistically significant in patients with DM vs those without DM in males and in females. Model 4 shows that after a further control of the effect of hypertension, the HRs were attenuated compared to Model 3, specifically the impact of DM on the risk of death from CBVD became a borderline significance in males ( $P = 0.06$ ). Finally, we excluded those who had heart disease and stroke at baseline (Model 5), the results show that HRs were further attenuated, except for a slight but non-significant increase in HR for death from CBVD in females.

Females appeared to have a higher HRs of DM for mortality from all-cause, heart disease and CVD than males. However, the increased HRs in females became non-significant after adjusting for age, race/ethnicity and education (Model 2), adjusting for behavior factors (Model 3), adjusting hypertension (Model 4), and excluding those who had heart disease and stroke at baseline (Model 5).

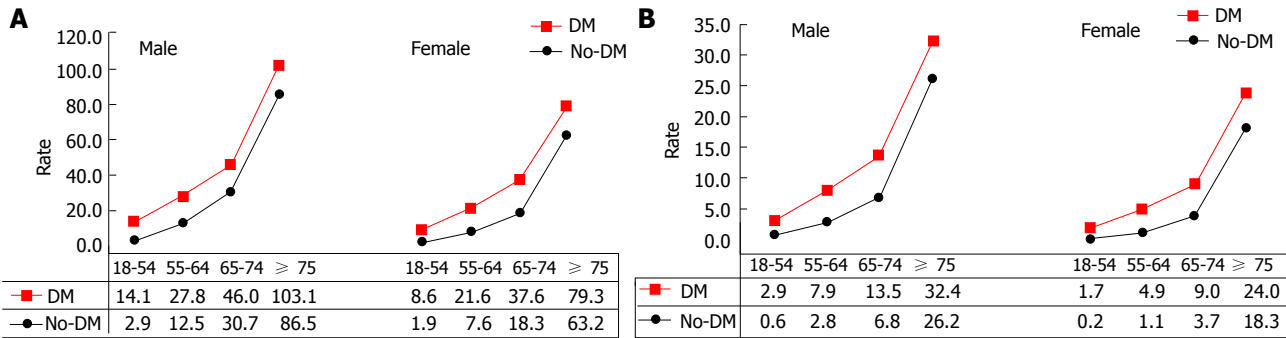
### Proportions of antidiabetic medication usage in patients with DM

Table 4 shows that in males, 10% of patients with DM did not use any antidiabetic medications, and 38.1% of DM patients used antidiabetic monotherapy, and 51.9% used ≥ 2 antidiabetic medications. The corresponding values of the prevalence of those who did not use any antidiabetic medication, those who used 1 only (*i.e.*, monotherapy), and those who used ≥ 2 were 10.3%, 40.4% and 49.4% in females respectively. Of those with monotherapy in males, 37.2% patients used insulin, followed by metformin (27.3%), sulfonylureas (25.9%) and others (9.6%). The most common three monotherapies in females were metformin (33.2%), insulin (33.0%), and sulfonylureas (21.9%), (gender differences:  $P = 0.008$ ). Among patients with combined antidiabetic medication therapies, the most frequent combination was metformin and sulfonylureas (20.1% in males, and 21.9% in females). No significant

**Table 2** Mortality (per 1000 person year) from all-cause, heart disease, cerebrovascular disease and cardiovascular disease by December 31, 2011 for baseline participants with or without diabetes, United States National Health Interview Surveys 2000-2009

		Male					Female				
		Non-DM		DM		P value	Non-DM		DM		P value
		Event	Rate	Event	Rate		Event	Rate	Event	Rate	
All-cause	No. of death	8363		2278			9485		2450		
Age	18-54.9	1765	2.9	317	14.1	< 0.0001	1384	1.9	249	8.6	< 0.0001
	55-64.9	1271	12.5	473	27.8	< 0.0001	989	7.6	403	21.6	< 0.0001
	65-74.9	1874	30.7	677	46.0	< 0.0001	1681	18.3	693	37.6	< 0.0001
	≥ 75	3453	86.5	811	103.1	< 0.0001	5431	63.2	1105	79.3	< 0.0001
Heart disease	No. of death	1707		543			1603		506		
Age	18-54.9	288	0.5	62	2.8	< 0.0001	115	0.2	41	1.4	< 0.0001
	55-64.9	244	2.4	116	6.8	< 0.0001	108	0.8	72	3.9	< 0.0001
	65-74.9	350	5.7	164	11.1	< 0.0001	241	2.6	132	7.2	< 0.0001
	≥ 75	825	20.7	201	25.6	0.032	1139	13.2	261	18.7	< 0.0001
CBVD	No. of death	376		112			621		134		
Age	18-54.9	50	0.1	4	0.2	0.063	58	0.1	8	0.3	0.002
	55-64.9	38	0.4	19	1.1	< 0.001	33	0.3	19	1.0	< 0.0001
	65-74.9	67	1.1	35	2.4	< 0.001	96	1.0	34	1.8	0.003
	≥ 75	221	5.5	54	6.9	0.694	434	5.0	73	5.2	0.371
CVD	No. of death	2083		655			2224		640		
Age	18-54.9	338	0.6	66	2.9	< 0.0001	173	0.2	49	1.7	< 0.0001
	55-64.9	282	2.8	135	7.9	< 0.0001	141	1.1	91	4.9	< 0.0001
	65-74.9	417	6.8	199	13.5	< 0.0001	337	3.7	166	9.0	< 0.0001
	≥ 75	1046	26.2	255	32.4	0.038	1573	18.3	334	24.0	< 0.0001

P values were given by  $\chi^2$  test. Mortality rates (per 1000) were estimated using Poisson regression. CBVD: Cerebrovascular disease; CVD: Cardiovascular disease; DM: Diabetes mellitus.



**Figure 3** Mortality from all-cause and cardiovascular disease by diabetes status. A: Mortality (per 1000 person year) from all-cause in patients without and with diabetes, United States NHIS 2000-2009; B: Mortality (per 1000 person year) from CVD in patients with diabetes by age and gender, United States NHIS 2000-2009. CVD: Cardiovascular disease; NHIS: National Health Interview Surveys; DM: Diabetes mellitus.

differences in the proportions of combined therapies between males and females were observed ( $P = 0.42$ ).

**Hazard ratios of antidiabetic medication use for risk of mortality**

Table 5 shows that after adjustment for key covariates, patients with treatment of antidiabetic medication vs those without had 7% lower risk of mortality from all-cause (HR = 0.93, 95%CI: 0.73-1.18,  $P = 0.56$ , Model 2) and 4% lower risk from CVD (HR = 0.96, 95%CI: 0.60-1.54,  $P = 0.87$ , Model 2), although these associations did not reach statistical significance. However, DM patients with metformin monotherapy had

a significantly decreased risk of all-cause mortality (HR = 0.55, 95%CI: 0.38-0.80,  $P = 0.002$ , Model 2), but those with insulin monotherapy showed an increased risk of all-cause mortality (HR = 1.71, 95%CI: 1.31-2.24,  $P < 0.0001$ , Model 2). A protective but non-significant effect of the treatment of antidiabetic medications (except for sulfonylureas and insulin use) on CVD mortality was observed.

In patients with DM, a combination of metformin, sulfonylureas and thiazolidinedione showed a significantly reduced risk of all-cause mortality compared to those who did not use a combination therapy (HR = 0.43, 95%CI: 0.27-0.70,  $P = 0.001$ , Model 2 in Table 5).

**Table 3** Multivariate adjusted hazard ratios (HR, 95%CI) of diabetes mellitus for mortality from all-cause, heart disease, cerebrovascular disease and cardiovascular disease by gender

Mortality	Male (n = 119399)			Female (n = 152750)			Excess HR <sup>1</sup>	
	HR	(95%CI)	P value	HR	(95%CI)	P value	Rate, %	P value
DM vs non-DM								
Model 1								
All-cause	1.56	(1.49-1.64)	< 0.0001	1.69	(1.61-1.78)	< 0.0001	8.3	0.02
Heart Dis	1.72	(1.53-1.93)	< 0.0001	2.02	(1.81-2.25)	< 0.0001	17.4	0.05
CBVD	1.48	(1.18-1.85)	0.001	1.43	(1.15-1.77)	0.001	-3.5	0.35
CVD	1.67	(1.51-1.86)	< 0.0001	1.85	(1.69-2.03)	< 0.0001	10.5	0.16
Model 2								
All-cause	1.54	(1.47-1.62)	< 0.0001	1.62	(1.55-1.71)	< 0.0001	5.5	0.13
Heart Dis	1.70	(1.51-1.91)	< 0.0001	1.95	(1.74-2.17)	< 0.0001	14.5	0.09
CBVD	1.48	(1.18-1.86)	0.001	1.39	(1.12-1.72)	0.003	-6.2	0.70
CVD	1.66	(1.49-1.84)	< 0.0001	1.79	(1.63-1.96)	< 0.0001	7.8	0.29
Model 3								
All-cause	1.47	(1.39-1.55)	< 0.0001	1.55	(1.47-1.63)	< 0.0001	5.5	0.15
Heart Dis	1.62	(1.44-1.82)	< 0.0001	1.80	(1.60-2.03)	< 0.0001	11.2	0.21
CBVD	1.35	(1.04-1.75)	0.023	1.40	(1.12-1.75)	0.003	3.4	0.85
CVD	1.57	(1.40-1.75)	< 0.0001	1.68	(1.52-1.86)	< 0.0001	7.4	0.35
Model 4								
All-cause	1.42	(1.35-1.49)	< 0.0001	1.50	(1.42-1.58)	< 0.0001	5.6	0.15
Heart Dis	1.58	(1.33-1.69)	< 0.0001	1.65	(1.48-1.89)	< 0.0001	4.4	0.19
CBVD	1.28	(0.99-1.66)	0.06	1.33	(1.06-1.66)	0.013	3.9	0.83
CVD	1.46	(1.31-1.63)	< 0.0001	1.58	(1.42-1.75)	< 0.0001	8.2	0.32
Model 5 - in those without baseline CVD								
All-cause	1.32	(1.23-1.41)	< 0.0001	1.40	(1.32-1.50)	< 0.0001	6.1	0.19
Heart Dis	1.22	(1.03-1.45)	0.019	1.43	(1.21-1.69)	< 0.0001	17.2	0.19
CBVD	1.24	(0.93-1.67)	0.137	1.41	(1.10-1.82)	0.008	13.7	0.53
CVD	1.22	(1.04-1.43)	0.017	1.45	(1.26-1.67)	< 0.0001	18.9	0.08

<sup>1</sup>Excess HR = [(HR in female/HR in male) - 1] × 100. Significant test was estimated based on sex × DM interaction. P-values were given by Cox regression models. Model 1: Adjusted for age and race/ethnicity (NH-White, NH-Black, Hispanics, and all other race group); Model 2: Adjusted for Model 1 plus education; Model 3: Adjusted for Model 2 plus smoking, alcohol, and physical activity status; Model 4: Adjusted for Model 2 plus smoking, alcohol, physical activity status, hypertension. DM: Diabetes mellitus; CBVD: Cerebrovascular disease; CVD: Cardiovascular disease.

A significantly reduced risk of all-cause mortality was observed as well in patients with any other combined drug therapies (0.68, 0.50-0.93,  $P = 0.016$ , Model 2). No significant association between combination medication use and risk of CVD mortality was observed for DM patients with or without combination therapies, except for those with thiazolidinedione plus any other antidiabetic medications (excluding insulin) (0.52, 0.28-0.98,  $P = 0.042$ ).

Table 6 shows that DM patients with insulin monotherapy showed an increased risk of heart disease mortality than those without insulin monotherapy (1.91, 1.12-3.26,  $P = 0.018$ , Model 2). A combination of metformin, sulfonylureas and insulin was significantly and negatively associated with heart disease mortality (0.35, 0.20-0.62,  $P < 0.0001$ , Model 2). No statistical significance in mortality from CBVD was observed in DM patients with or without medication (neither monotherapy nor combinations).

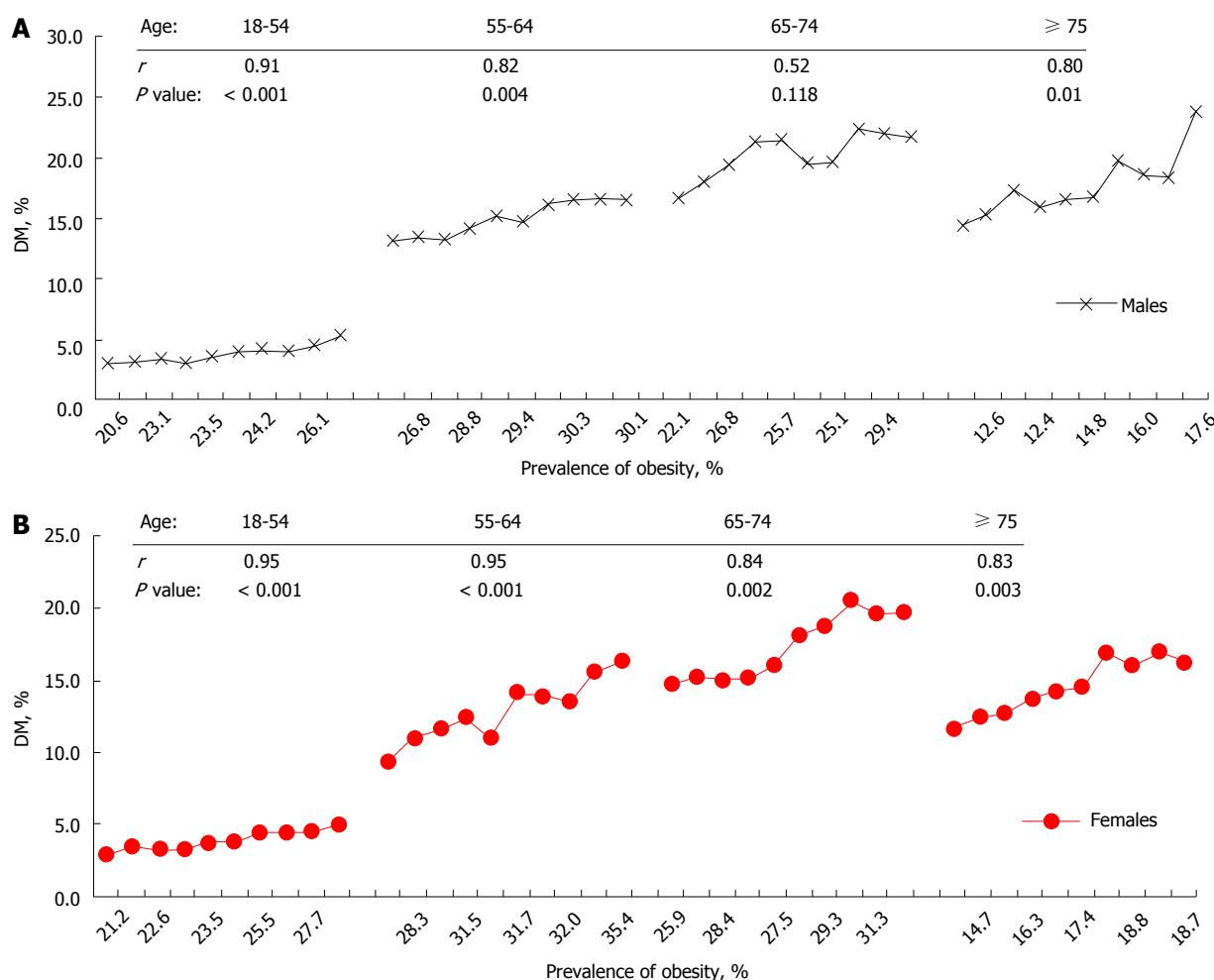
## DISCUSSION

The present study, using data from one of the largest national health survey systems and multiple linkage files, examined the burden of DM and its impact on CVD and all-cause mortality among adults in the United

States. The study adds new evidence to the body of scientific literatures regarding antidiabetic medication profiles and health outcomes in patients with DM. The main findings show that: (1) the prevalence of DM significantly increased in all age groups for males and females in the last decade; (2) patients with DM had 1.47 to 1.62 times higher risk of death from all-cause and CVD in males, and 1.55 to 1.68 times higher in females compared to those without DM; (3) about 40% of patients with DM used antidiabetic monotherapy, and about 50% used combined antidiabetic therapy, however 10% of patient with DM did not use any medication in both males and females; and (4) in patients with DM, using metformin monotherapy or a combined therapy of metformin with other antidiabetic medications showed a significantly reduced risk of all-cause mortality. This protective association remained significant after adjustment for age, sex, race/ethnicity, survey year, antihypertensive drug, and anti-dyslipidemia medication use.

The present study confirmed an increased prevalence of DM in the last decade in the United States. This finding is consistent with previous reports<sup>[1-4]</sup>, and provides new evidence at the national level. Several factors may contribute to the increased rates. Of them, an increased prevalence of obesity across the





**Figure 4** Changes in obesity rates and its correlation with diabetes rates. A: Correlation between prevalence of obesity and diabetes by age in males, United States NHIS 2000-2009; B: Correlation between prevalence of obesity and diabetes by age in females, United States NHIS 2000-2009. NHIS: National Health Interview Surveys; DM: Diabetes mellitus.

nation may contribute to the increased trend of DM. We analyzed obesity rate using the same NHIS data. Figure 4 depicts a positive correlation trend between the prevalence of obesity and DM between 2000 and 2009 by four age groups in males (Figure 4A) and in females (Figure 4B) between 2000 and 2009. The highest correlation coefficient (*r*) was shown in ages 18-54 (*r* = 0.91, *P* < 0.001) in males, and ages 18-54 (*r* = 0.95, *P* < 0.001), and 55-64 (*r* = 0.95, *P* < 0.001) in females. Given the well-known pathophysiological mechanisms of obesity and risk of DM, this finding suggests that control of obesity would play a pivotal role in stopping the unwelcome trends of DM. In addition, females aged 55-74 have a greater increased trend of DM than males (Figure 1B). Although it is unclear why there is a notable increase in this age group for females, changes in female hormone at pre- and post-menopausal ages may partly explain this gender difference in the risk of DM and other chronic diseases<sup>[5,7,17-21]</sup>. Data from the Women's Health Initiative Hormone Trial suggest that combined therapy with estrogen and progestin reduces the incidence of DM<sup>[21]</sup>.

As demonstrated in several studies, we observed

an excess relative risk (*i.e.*, hazard ratio) of DM for all-cause and heart disease in females vs males. However, this excess risk became non-significant after adjustment for key covariates. Findings using data from the earlier Framingham Heart Study (FHS) surveys (1970s and 1980s) demonstrated a significant excess risk of recurrent myocardial infarction and fatal coronary heart disease for women with DM vs men with DM<sup>[22,23]</sup>. Our non-significant results are not consistent with the previous report. It may be attributable to the different datasets we used from the FHS. For example, the majority of participants in FHS were white middle class individuals who may have different risk profiles from minorities and people with lower social status. Furthermore, a decreased relative risk of DM for CVD in recent generations has been observed because of early diagnosis and disease prevention as well as more advanced treatment than two or three decades ago. Nevertheless, this relatively higher risk of DM for coronary heart disease in women vs men should be still taken into consideration in CVD risk assessment and disease prevention. In the study, among 5 preventable CVD risk factors that we examined, 4 (percent of

**Table 4 Proportion of antidiabetic medication use in patients with diabetes by gender**

		Male		Female		P value
		%	(SEP)	%	(SEP)	
By groups						
	Monotherapy	38.11	(1.19)	40.36	(1.06)	0.291
	Combination	51.89	(1.27)	49.38	(1.10)	
	No drug	10.00	(0.83)	10.26	(0.66)	
Monotherapy						
	Biguanides (Metformin)	27.32	(1.89)	33.24	(1.57)	0.008
	SU	25.86	(1.72)	21.88	(1.34)	
	Insulin	37.20	(1.85)	33.01	(1.42)	
	Others	9.63	(1.16)	11.87	(0.95)	
Combination						
	Metformin + SU	20.05	(1.34)	21.94	(1.10)	0.422
	TZD + Any (insulin excluded)	13.56	(1.25)	13.11	(0.87)	
	Insulin + Any (TZD excluded)	15.25	(1.38)	16.91	(1.07)	
	Metformin + SU + TZD	7.72	(0.86)	6.75	(0.69)	
	Metformin + SU + Insulin	6.89	(0.87)	4.97	(0.61)	
	Any other combinations	36.52	(1.85)	36.32	(1.44)	

P values were given by  $\chi^2$  test. Others include alpha glucosidase inhibitors, meglitinides, dipeptidyl peptidase 4 inhibitors, amylin analogs, and incretin mimetics. TZD: Thiazolidinedione; SU: Sulfonylureas; SEP: Standard error of proportion.

**Table 5 Multivariate adjusted hazard ratios (HR, 95%CI) of antidiabetic medication use for mortality from all-cause and cardiovascular disease in patients with diabetes**

	All-cause mortality						Mortality from CVD					
	Model 1			Model 2			Model 1			Model 2		
	HR	(95%CI)	P value	HR	(95%CI)	P value	HR	(95%CI)	P value	HR	(95%CI)	P value
Medication use <i>vs</i> no-use	0.87	(0.68-1.11)	0.251	0.93	(0.73-1.18)	0.556	0.88	(0.54-1.44)	0.613	0.96	(0.60-1.54)	0.873
Monotherapy (ref: Non-drug use)												
Biguanides (Metformin)	0.53	(0.36-0.77)	0.001	0.55	(0.38-0.80)	0.002	0.82	(0.42-1.61)	0.564	0.87	(0.45-1.68)	0.681
SU	0.89	(0.66-1.21)	0.456	0.91	(0.67-1.23)	0.529	1.10	(0.66-1.83)	0.716	1.11	(0.67-1.84)	0.696
Insulin	1.65	(1.26-2.16)	< 0.001	1.71	(1.31-2.24)	< 0.0001	1.51	(0.86-2.66)	0.153	1.58	(0.92-2.70)	0.094
Combination												
Metformin + SU	0.75	(0.55-1.01)	0.059	0.81	(0.60-1.10)	0.168	0.77	(0.41-1.43)	0.403	0.87	(0.48-1.57)	0.632
TZD + Any (insulin excluded)	0.87	(0.60-1.26)	0.468	0.98	(0.67-1.42)	0.905	0.43	(0.23-0.82)	0.011	0.52	(0.28-0.98)	0.042
Insulin + Any (TZD excluded)	1.27	(0.90-1.78)	0.171	1.33	(0.94-1.86)	0.103	1.37	(0.69-2.72)	0.365	1.45	(0.75-2.81)	0.264
Metformin + SU + TZD	0.40	(0.25-0.65)	< 0.001	0.43	(0.27-0.70)	0.001	0.54	(0.24-1.18)	0.120	0.58	(0.27-1.25)	0.164
Metformin + SU + Insulin	0.64	(0.41-1.01)	0.053	0.67	(0.43-1.05)	0.080	0.75	(0.44-1.29)	0.293	0.80	(0.47-1.37)	0.418
Other combination	0.63	(0.46-0.86)	0.004	0.68	(0.50-0.93)	0.016	0.59	(0.31-1.09)	0.091	0.64	(0.35-1.19)	0.157

Others: Include alpha glucosidase inhibitors, meglitinides, dipeptidyl peptidase 4 inhibitors, amylin analogs, and incretin mimetics. P values were given by Cox regression models. Model 1: Adjusted for age, sex, race/ethnicity, and survey year; Model 2: Adjusted for Model 1 plus anti-hypertension and anti-hyperlipidemia drugs. TZD: Thiazolidinedione; SU: Sulfonylureas.

individuals with lower socioeconomic status, assessed by education level, the proportion of individuals who were physically inactive, the proportion of individuals with obesity, and the proportion of individuals who had hypertension) were significantly higher in females than males, although males had a higher smoking rate than females (Table 7). These risk factors differences may partly explain the relative risk difference between genders. It is clear further studies are needed to assess the gender differences, including studies of the

established and emerging risk predictors<sup>[15,24-28]</sup>.

The present study provides new evidence of the patterns of antidiabetic medicine usage and their impact on all-cause and CVD mortalities in patients with DM. Treatments with metformin, insulin, and sulfonylureas were the top three medications in the study population. More than one third of patients took insulin, which is commonly given to patients either for a short-term use because of significantly out of control serum glucose, or for long-term glucose control because

**Table 6** Multivariate adjusted hazard ratios (HR, 95%CI) of antidiabetic medication use for mortality from heart disease and cerebrovascular disease in patients with diabetes

	Heart disease mortality						Cerebrovascular disease (CBVD) mortality					
	Model 1			Model 2			Model 1			Model 2		
	HR	(95%CI)	P value	HR	(95%CI)	P value	HR	(95%CI)	P value	HR	(95%CI)	P value
Medication use <i>vs</i> no-use	1.01	(0.62-1.65)	0.982	1.08	(0.67-1.74)	0.741	0.58	(0.19-1.78)	0.339	0.67	(0.23-1.95)	0.457
Monotherapy												
Biguanides (Metformin)	0.80	(0.38-1.67)	0.550	0.83	(0.40-1.70)	0.608	0.94	(0.22-4.01)	0.938	1.20	(0.30-4.82)	0.792
SU	1.26	(0.76-2.11)	0.373	1.27	(0.76-2.13)	0.364	0.71	(0.23-2.24)	0.560	0.76	(0.25-2.34)	0.629
Insulin	1.85	(1.06-3.24)	0.031	1.91	(1.12-3.26)	0.018	0.60	(0.16-2.23)	0.445	0.71	(0.23-2.21)	0.547
Combined												
Metformin + SU	0.82	(0.41-1.67)	0.586	0.91	(0.47-1.80)	0.794	0.59	(0.14-2.47)	0.472	0.71	(0.17-2.94)	0.632
TZD + Any (insulin excluded)	0.55	(0.28-1.08)	0.082	0.68	(0.35-1.30)	0.242	0.14	(0.02-1.18)	0.070	0.15	(0.02-1.32)	0.088
Insulin + Any (TZD excluded)	1.79	(0.89-3.61)	0.103	1.85	(0.94-3.64)	0.074	0.44	(0.13-1.53)	0.196	0.50	(0.15-1.66)	0.257
Metformin + SU + TZD	0.61	(0.27-1.40)	0.245	0.65	(0.30-1.42)	0.280	0.34	(0.04-2.83)	0.316	0.41	(0.04-3.95)	0.438
Metformin + SU + Insulin	0.33	(0.19-0.59)	0.000	0.35	(0.20-0.62)	< 0.0001	1.66	(0.55-5.02)	0.368	1.91	(0.64-5.73)	0.245
Other combinations	0.71	(0.37-1.37)	0.307	0.78	(0.42-1.47)	0.444	0.28	(0.06-1.31)	0.106	0.31	(0.07-1.41)	0.129

Others: Include alpha glucosidase inhibitors, meglinitides, dipeptidyl peptidase 4 inhibitors, amylin analogs, and incretin mimetics. *P* values were given by Cox regression models. Model 1: Adjusted for age, sex, race/ethnicity, and survey year; Model 2: Adjusted for Model 1 plus anti-hypertension and anti-hyperlipidemia drugs. TZD: Thiazolidinedione; SU: Sulfonylureas.

their DM has progressed over many years (commonly between 10 and 20 years) and their pancreas can no longer make enough insulin to respond to other glucose-lowering medications<sup>[5,29]</sup>. Similar to previous studies, findings from the present study suggest a significant protective effect on all-cause mortality, and a protective effect on CVD mortality for those using metformin or metformin combined with other glucose-lowering medications. Metformin, a class of medications known as “biguanides” and a first-line agent for type 2 DM (T2DM) pharmacotherapy, is one of the most prescribed drugs worldwide<sup>[30]</sup>. It has been suggested that the potential mechanisms by which metformin reduces the risk of mortality is lowering blood glucose by reducing hepatic glucose output, decreasing intestinal glucose absorption, and controlling body weight by decreasing food intake<sup>[30-32]</sup>. The mechanism of the cardiovascular effect of metformin was reported to improve lipoprotein profiles in diabetic patients by decreasing plasma concentrations of free fatty acid, triglycerides, total cholesterol and LDL cholesterol and increased HDL cholesterol<sup>[30]</sup>. Meanwhile, all-cause mortality includes deaths from cancer as well. Several studies have shown a significant risk reduction in cancer incidence and mortality among diabetic patients on metformin use relative to other antidiabetic drugs use<sup>[33]</sup>. Furthermore, in considering that biguanides demonstrate a better safety profile than most oncology drugs in current anticancer drug use, nonconventional routes for administering diabetobiguanides for cancer treatment has been suggested<sup>[34]</sup>. Findings from the present study support the current knowledge of metformin therapy in the reduction of CVD and total mortality, although further studies are needed in detail on its specific association with CVD and cancers.

In addition to the strength of using a large-scale sample size, the present study has several other advan-

tages. First, using the NHIS-Mortality Linked Files, we were able to test the association between DM and risk of outcomes prospectively. Second, by using NHIS-MEPS linkage Files, we were able to test the patterns of medications which paves the way for us to further test more details on the association between pharmacotherapy and disease outcomes using a nationally representative dataset.

Similar to any study, however, the present study has several limitations. First, we were unable to classify whether a patient with DM was type 1 DM (T1DM) or T2DM because the NHIS data did not collect the information. Therefore, findings from the study cannot be applied to interpret risk differences between T1DM and T2DM. However, although T1DM can occur at any age, it is most often diagnosed in children, adolescents, or young adults. The NHIS’s participants were aged 18 and older. Furthermore, it is well-known that the majority of total DM are T2DM in general population, we may be able to assume the majority DM cases in the NHIS data were T2DM. Second, baseline predictors were measured once only, that any changes in the study variables after baseline may affect the prospective estimates of the associations between baseline predictors and health outcomes. Findings of the study should be on the basis of the hypothesis that these changes, if any, were randomized across all participants, so that a potential time-varying bias would be small when a study uses a large-scale sample size<sup>[35]</sup>. Third, participants’ medical conditions at baseline were self-reported physician-diagnosis of disease (hypertension, CHD, stroke and DM), therefore possible recall bias may occur. However, the recall bias might have a relatively small effect, because the use of self-reports of physician-diagnosis of disease have been confirmed as a valid approach in large-scale population health surveys in the United States<sup>[36,37]</sup>. Fourth, the NHIS did not have data

**Table 7** Age-race-adjusted rates of five selected predictors for cardiovascular disease in patients with diabetes at baseline, 2000-2009 National Health Interview Surveys

	Patients with DM at baseline				
	Male ( <i>n</i> = 9892)		Female ( <i>n</i> = 12413)		<i>P</i> value
	Rate	(SEP)	Rate	(SEP)	
Education					
Less than HS	21.53	(0.50)	24.82	(0.47)	< 0.0001
HS Graduated	28.44	(0.59)	30.61	(0.55)	0.0100
≥ College	50.03	(0.60)	44.57	(0.56)	< 0.0001
Smoking status					
No smoker	40.92	(0.58)	61.60	(0.53)	< 0.0001
Former smoker	36.03	(0.58)	17.81	(0.49)	< 0.0001
Current smoker	23.05	(0.46)	20.59	(0.35)	< 0.0001
Exercise					
Inactive	42.80	(0.63)	49.34	(0.62)	< 0.0001
Insufficiently active	26.88	(0.52)	27.98	(0.49)	0.1000
Sufficiently active	30.32	(0.61)	22.68	(0.47)	< 0.0001
BMI, kg/m <sup>2</sup>					
Overweight	34.21	(0.55)	25.82	(0.46)	< 0.0001
Obesity	47.13	(0.60)	53.88	(0.51)	< 0.0001
Medical condition					
Hypertension	52.54	(0.58)	54.55	(0.46)	0.0100

Education ≥ College including those with associate degrees. Overweight: Body mass index (BMI) 25 to 29.9 kg/m<sup>2</sup>. Obesity: BMI ≥ 30 kg/m<sup>2</sup>. *P* values were given by  $\chi^2$  test. HS: High school.

on participants' physical exams and laboratory tests (*i.e.*, without exact blood pressure measures, and measures from serum lipids and metabolic biomarkers), which may not only lead to underestimate the prevalence of hypertension and DM, but also limit us to quantitatively estimate the association between antidiabetic drug use and changes in serum HbA1c (a biomarker of glycaemia control status in diabetic patients) and lipid profiles, and their impacts on the study outcomes. Therefore, the findings of the study provide a relatively conservative estimate of the burdens of disease. Fifth, we were unable to test subgroups of antidiabetic drugs' effects on the study outcomes, such as the subgroups of sulfonylureas, because the detail data was not available from NHIS-MESP Linkage File. Sixth, in multivariate analysis, we cannot always be able to control adequately for confounding factors. We may not even know about them and chance cannot be discarded although it is highly unlikely.

Despite the limitations discussed the above, three clear and important conclusions follow the present study. First, the prevalence of DM significantly increased in all age groups in the past decade, with specific increase in females aged 55-74 compared to males. Second, DM is a significant predictor for mortality from all-cause and CVD in both genders, with a slightly higher excess relative risk in females vs males. Third, about 10% of patients with DM do not receive antidiabetic therapy. DM patients who received metformin monotherapy or combination of metformin with other antidiabetic medications (except insulin) showed a significant protective effect on all-cause mortality.

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## COMMENTS

### Background

Diabetes mellitus (DM) is a leading cause of death in the United States. The present study examined the trend of DM using data from nationally representative surveys from 2000 to 2009. It is one of the first studies that address the epidemic of DM in the nation, and its serious impact on population health. Furthermore, findings from the study add new evidence of glucose-lowering treatment and health outcomes in patients with DM using data from the real world, instead of using a sample from very selective participants that are commonly applied in clinical trials.

### Research frontiers

The amount of data in the real world has been exploding, and analyzing large-scale data sets, so called Big data is becoming a key basis of competition and productivity in epidemiological studies of DM. The present study using a large-scale dataset from multiple sources not only addresses the epidemic of DM in the United States, but also advances the research methods to build a national cohort sample by taking the advantages of national health survey data at baseline linking health medication prescription and vital statistics for a decade time period. Findings from this approach provide a unique opportunity to address drug effects on health outcomes using data from real world.

### Innovations and breakthroughs

The innovations of the study are characterized by its research design linking data from multiple sources, and building up a representative sample of national cohort study.

### Applications

The study design adds new research approach to the body of study designs using data from population based studies. Findings from the study are very informative for counties that are experiencing an increasing trend of obesity and diabetes in the world.

### Terminology

Cross-sectional study design; Prospective study design; Linked Files.

### Peer-review

This is a timely, interesting and informative report.

## REFERENCES

- 1 Association AD. Statistics About Diabetes. [accessed 2014 Jan 15]. Available from: URL: <http://www.diabetes.org/diabetes-basics/statistics/>
- 2 Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004; **27**: 1047-1053 [PMID: 15111519 DOI: 10.2337/diacare.27.5.1047]
- 3 Liu L, Yin X, Morrissey S. Global variability in diabetes mellitus



- and its association with body weight and primary healthcare support in 49 low- and middle-income developing countries. *Diabet Med* 2012; **29**: 995-1002 [PMID: 22150805 DOI: 10.1111/j.1464-5491.2011.03549.x]
- 4 **Meeto D**, McGovern P, Safadi R. An epidemiological overview of diabetes across the world. *Br J Nurs* 2007; **16**: 1002-1007 [PMID: 18026039 DOI: 10.12968/bjon.2007.16.16.27079]
- 5 **Beckman JA**, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. *JAMA* 2002; **287**: 2570-2581 [PMID: 12020339 DOI: 10.1001/jama.287.19.2570]
- 6 **Berry C**, Tardif JC, Bourassa MG. Coronary heart disease in patients with diabetes: part I: recent advances in prevention and noninvasive management. *J Am Coll Cardiol* 2007; **49**: 631-642 [PMID: 17291928 DOI: 10.1016/j.jacc.2006.09.046]
- 7 **Sarwar N**, Gao P, Seshasai SR, Gobin R, Kaptoge S, Di Angelantonio E, Ingelsson E, Lawlor DA, Selvin E, Stampfer M, Stehouwer CD, Lewington S, Pennells L, Thompson A, Sattar N, White IR, Ray KK, Danesh J. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. *Lancet* 2010; **375**: 2215-2222 [PMID: 20609967 DOI: 10.1016/S0140-6736(10)60484-9]
- 8 **Rutter MK**, Meigs JB, Sullivan LM, D'Agostino RB, Wilson PW. Insulin resistance, the metabolic syndrome, and incident cardiovascular events in the Framingham Offspring Study. *Diabetes* 2005; **54**: 3252-3257 [PMID: 16249452 DOI: 10.2337/diabetes.54.11.3252]
- 9 **Leon BM**, Maddox TM. Diabetes and cardiovascular disease: Epidemiology, biological mechanisms, treatment recommendations and future research. *World J Diabetes* 2015; **6**: 1246-1258 [PMID: 26468341 DOI: 10.4239/wjcd.v6.i13.1246]
- 10 **NCHS**. Leading Causes of Death. [updated 2014 Jun 8]. Available from: URL: <http://www.cdc.gov/nchs/fastats/leading-causes-of-death.htm>
- 11 **Wong ND**, Glovaci D, Wong K, Malik S, Franklin SS, Wygant G, Iloeje U. Global cardiovascular disease risk assessment in United States adults with diabetes. *Diab Vasc Dis Res* 2012; **9**: 146-152 [PMID: 22377485 DOI: 10.1177/1479164112436403]
- 12 **Centers for Disease Control and Prevention (CDC)**. Increasing prevalence of diagnosed diabetes--United States and Puerto Rico, 1995-2010. *MMWR Morb Mortal Wkly Rep* 2012; **61**: 918-921 [PMID: 23151951]
- 13 **Menke A**, Casagrande S, Geiss L, Cowie CC. Prevalence of and Trends in Diabetes Among Adults in the United States, 1988-2012. *JAMA* 2015; **314**: 1021-1029 [PMID: 26348752 DOI: 10.1001/jama.2015.10029]
- 14 **NCHS-NHIS**. National Health Interview Survey. Available from: URL: <http://www.cdc.gov/nchs/nhis.htm>
- 15 **Liu L**, Núñez AE, An Y, Liu H, Chen M, Ma J, Chou EY, Chen Z, Eisen HJ. Burden of Cardiovascular Disease among Multi-Racial and Ethnic Populations in the United States: an Update from the National Health Interview Surveys. *Front Cardiovasc Med* 2014; **1**: 8 [PMID: 26664859 DOI: 10.3389/fcvm.2014.00008]
- 16 **AHRQ-MEPS**. Medical Expenditure Panel Survey. Available from: URL: <http://meps.ahrq.gov/mepsweb/>
- 17 **Butler J**, Rodondi N, Zhu Y, Figaro K, Fazio S, Vaughan DE, Satterfield S, Newman AB, Goodpaster B, Bauer DC, Holvoet P, Harris TB, de Rekeneire N, Rubin S, Ding J, Kritchevsky SB. Metabolic syndrome and the risk of cardiovascular disease in older adults. *J Am Coll Cardiol* 2006; **47**: 1595-1602 [PMID: 16630996 DOI: 10.1016/j.jacc.2005.12.046]
- 18 **Best LE**, Hayward MD, Hidayat MM. Life course pathways to adult-onset diabetes. *Soc Biol* 2005; **52**: 94-111 [PMID: 17619606 DOI: 10.1080/19485565.2005.9989104]
- 19 **Yamori Y**, Liu L, Ikeda K, Mizushima S, Nara Y, Simpson FO. Different associations of blood pressure with 24-hour urinary sodium excretion among pre- and post-menopausal women. WHO Cardiovascular Diseases and Alimentary Comparison (WHO-CARDIAC) Study. *J Hypertens* 2001; **19**: 535-538 [PMID: 11327626 DOI: 10.1097/00004872-200103001-00003]
- 20 **Carr MC**. The emergence of the metabolic syndrome with menopause. *J Clin Endocrinol Metab* 2003; **88**: 2404-2411 [PMID: 12788835 DOI: 10.1210/jc.2003-030242]
- 21 **Margolis KL**, Bonds DE, Rodabough RJ, Tinker L, Phillips LS, Allen C, Bassford T, Burke G, Torrens J, Howard BV. Effect of oestrogen plus progestin on the incidence of diabetes in postmenopausal women: results from the Women's Health Initiative Hormone Trial. *Diabetologia* 2004; **47**: 1175-1187 [PMID: 15252707 DOI: 10.1007/s00125-004-1448-x]
- 22 **Abbott RD**, Donahue RP, Kannel WB, Wilson PW. The impact of diabetes on survival following myocardial infarction in men vs women. The Framingham Study. *JAMA* 1988; **260**: 3456-3460 [PMID: 2974889 DOI: 10.1001/jama.1988.03410230074031]
- 23 **Kannel WB**, McGee DL. Diabetes and cardiovascular disease. The Framingham study. *JAMA* 1979; **241**: 2035-2038 [PMID: 430798 DOI: 10.1001/jama.1979.03290450033020]
- 24 **Kabadi SM**, Lee BK, Liu L. Joint effects of obesity and vitamin D insufficiency on insulin resistance and type 2 diabetes: results from the NHANES 2001-2006. *Diabetes Care* 2012; **35**: 2048-2054 [PMID: 22751957 DOI: 10.2337/dc12-0235]
- 25 **Liu L**, Chen M, Hankins SR, Núñez AE, Watson RA, Weinstock PJ, Newschaffer CJ, Eisen HJ. Serum 25-hydroxyvitamin D concentration and mortality from heart failure and cardiovascular disease, and premature mortality from all-cause in United States adults. *Am J Cardiol* 2012; **110**: 834-839 [PMID: 22658246 DOI: 10.1016/j.amjcard.2012.05.013]
- 26 **Liu L**. Joint Effects of Serum 25 (OH) D and C-Reactive Protein Concentration on Coronary Heart Disease and All-cause Mortality in Patients with Diabetes Mellitus. *J Heart Health* 2015; **1** [DOI: 10.16966/2379-769X.105]
- 27 **Liu L**, Nettleton JA, Bertoni AG, Bluemke DA, Lima JA, Szklo M. Dietary pattern, the metabolic syndrome, and left ventricular mass and systolic function: the Multi-Ethnic Study of Atherosclerosis. *Am J Clin Nutr* 2009; **90**: 362-368 [PMID: 19515735 DOI: 10.3945/ajcn.2009.27538]
- 28 **Sun J**, Rangan P, Bhat SS, Liu L. A Meta-Analysis of the Association between Helicobacter pylori Infection and Risk of Coronary Heart Disease from Published Prospective Studies. *Helicobacter* 2016; **21**: 11-23 [PMID: 25997465 DOI: 10.1111/hel.12234]
- 29 **Holman RR**, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-year follow-up of intensive glucose control in type 2 diabetes. *N Engl J Med* 2008; **359**: 1577-1589 [PMID: 18784090 DOI: 10.1056/NEJMoa0806470]
- 30 **Batchuluun B**, Sonoda N, Takayanagi R, Inoguchi T. The Cardiovascular Effects of Metformin: Conventional and New Insights. *J Endocrinol Diabetes Obes* 2014; **2**: 1035
- 31 **Stumvoll M**, Nurjhan N, Perriello G, Dailey G, Gerich JE. Metabolic effects of metformin in non-insulin-dependent diabetes mellitus. *N Engl J Med* 1995; **333**: 550-554 [PMID: 7623903 DOI: 10.1056/NEJM199508313330903]
- 32 **Lee A**, Morley JE. Metformin decreases food consumption and induces weight loss in subjects with obesity with type II non-insulin-dependent diabetes. *Obes Res* 1998; **6**: 47-53 [PMID: 9526970 DOI: 10.1002/j.1550-8528.1998.tb00314.x]
- 33 **DeCensi A**, Puntoni M, Guerrieri-Gonzaga A, Cazzaniga M, Serrano D, Lazzeroni M, Vingiani A, Gentilini O, Petrer M, Viale G, Cuzick J, Bonanni B, Pruneri G. Effect of Metformin on Breast Ductal Carcinoma In Situ Proliferation in a Randomized Presurgical Trial. *Cancer Prev Res (Phila)* 2015; **8**: 888-894 [PMID: 26276754 DOI: 10.1158/1940-6207.CAPR-15-0048]
- 34 **Menendez JA**, Quirantes-Piné R, Rodríguez-Gallego E, Cufi S, Corominas-Faja B, Cuyàs E, Bosch-Barrera J, Martín-Castillo B, Segura-Carretero A, Joven J. Oncobiguanides: Paracelsus' law and nonconventional routes for administering diabetobiguanides for cancer treatment. *Oncotarget* 2014; **5**: 2344-2348 [PMID: 24909934 DOI: 10.18632/oncotarget.1965]
- 35 **Szklo M**, Nieto FJ. Epidemiology Beyond the Basics. Sudbury, MA: Jones and Bartlett, 2007
- 36 **Glymour MM**, Avendano M. Can self-reported strokes be used to study stroke incidence and risk factors?: evidence from the health

and retirement study. *Stroke* 2009; **40**: 873-879 [PMID: 19150869  
DOI: 10.1161/STROKEAHA.108.529479]

37 **Bush TL**, Miller SR, Golden AL, Hale WE. Self-report and medical

record report agreement of selected medical conditions in the  
elderly. *Am J Public Health* 1989; **79**: 1554-1556 [PMID: 2817172  
DOI: 10.2105/AJPH.79.11.1554]

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Observational Study

## Cost of illness among patients with diabetic foot ulcer in Turkey

Ergun Oksuz, Simten Malhan, Bilge Sonmez, Rukiye Numanoglu Tekin

Ergun Oksuz, Department of Family Medicine, Faculty of Medicine, Baskent University, 06770 Ankara, Turkey

Simten Malhan, Faculty of Health Sciences, Baskent University, 06770 Ankara, Turkey

Bilge Sonmez, Emir Karatekin Family Health Center, Cankiri Karatekin University, 18100 Cankiri, Turkey

Rukiye Numanoglu Tekin, Faculty of Health Sciences, Baskent University, 6770 Ankara, Turkey

Telephone: +90-312-2466773  
 Fax: +90-312-2466770

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**Correspondence to:** Dr. Ergun Oksuz, MD, Professor, Department of Family Medicine, Faculty of Medicine, Baskent University, Baglica Campus, 06770 Ankara, Turkey. [eoksuz@baskent.edu.tr](mailto:eoksuz@baskent.edu.tr)

### Abstract

#### AIM

To evaluate the annual cost of patients with Wagner grade 3-4-5 diabetic foot ulcer (DFU) from the public payer's perspective in Turkey.

#### METHODS

This study was conducted focused on a time frame of one year from the public payer's perspective. Cost-of-illness (COI) methodology, which was developed by the World Health Organization, was used in the generation of cost data. By following a clinical path with the COI method, the main total expenses were reached by multiplying the number of uses of each expense item, the percentage of cases that used them and unit costs. Clinical guidelines and real data specific to Turkey were used in the calculation of the direct costs. Monte Carlo Simulation was used in the study as a sensitivity analysis.

#### RESULTS

The following were calculated in DFU treatment from the public payer's perspective: The annual average per patient outpatient costs \$579.5 (4.1%), imaging test costs \$283.2 (2.0%), laboratory test costs \$284.8 (2.0%), annual average per patient cost of intervention, rehabilitation and trainings \$2291.7 (16.0%), annual average per patient cost of drugs used \$2545.8 (17.8%)

and annual average per patient cost of medical materials used in DFU treatment \$735.0 (5.1%). The average annual per patient cost for hospital admission is \$7357.4 (51.5%). The average per patient complication cost for DFU is \$210.3 (1.5%). The average annual per patient cost of DFU treatment in Turkey is \$14287.70. As a result of the sensitivity analysis, the standard deviation of the analysis was \$5706.60 ( $n = 5000$ , mean = \$14146.8, 95%CI: \$13988.6-\$14304.9).

### CONCLUSION

The health expenses per person are \$-PPP 1045 in 2014 in Turkey and the average annual per patient cost for DFU is 14-fold of said amount. The total health expense in 2014 in Turkey is \$-PPP 80.3 billion and the total DFU cost has a 3% share in the total annual health expenses for Turkey. Hospital costs are the highest component in DFU disease costs. In order to prevent DFU, training of the patients at risk and raising consciousness in patients with diabetes mellitus (DM) will provide benefits in terms of economy. Appropriate and efficient treatment of DM is a health intervention that can prevent complications.

**Key words:** Diabetic foot; Diabetes complications; Cost of illness; Burden of illness; Amputation

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**Core tip:** The purpose of this study is to evaluate the annual cost of patients with Wagner grade 3-4-5 diabetic foot ulcer (DFU) in Turkey. Cost-of-illness methodology was used in the generation of cost data. Monte Carlo Simulation was used in the study as a sensitivity analysis. The average annual per patient cost of DFU treatment in Turkey is \$14287.70. As a result of the sensitivity analysis, the standard deviation of the analysis was \$5706.60 ( $n = 5000$ , mean = \$14146.8, 95%CI: \$13988.6-\$14304.9). Hospital costs are the highest component in DFU disease costs.

Oksuz E, Malhan S, Sonmez B, Numanoglu Tekin R. Cost of illness among patients with diabetic foot ulcer in Turkey. *World J Diabetes* 2016; 7(18): 462-469 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v7/i18/462.htm> DOI: <http://dx.doi.org/10.4239/wjd.v7.i18.462>

### INTRODUCTION

Diabetes mellitus (DM) is a health problem, the severity of which is gradually increasing all over the world. DM exists in approximately 8.3% of the world's population. In 2013, 10.8% (\$548 billion) of global health expenses were for DM and its complications<sup>[1]</sup>. DM amounts to 23% (approximately 10 billion Turkish liras - TL) of the total health expenses of Turkey in 2012<sup>[2]</sup>.

Diabetic foot ulcer (DFU) is a frequently observed,

serious, and chronic complication of DM. The risk of occurrence during diagnosis can be up to 25%, and 2% of cases require amputation<sup>[3]</sup>. Half of the cases of non-traumatic foot amputation are due to DM<sup>[4]</sup>. It is estimated that a patient's foot is amputated due to DFU once every 30 s worldwide. The rate of recurring amputation is between 30% and 50% in the following three years in the patients who are amputated once. The rate of death within five years following amputation is 50%<sup>[5]</sup>.

Approximately 400000 DFU cases are observed in Turkey, and 7700 amputation procedures are performed annually due to DFU<sup>[6]</sup>. As the prognosis of cases after amputation is considered, the importance of rational treatment in DFU becomes significant. Surgical and non-surgical basic wound care principles are essential in the efficient recovery of the wounds. Prevention of ulcers and fighting against wound site infections that are difficult to heal are as important as its treatment. DFU treatment requires multidisciplinary treatment procedures. DFU has direct costs as well as indirect costs and it is very important to try to increase quality of life of the patients during treatment, minimize the disease costs, and administer correct treatment that enables the person to remain as a productive and value-adding individual as well as to prevent occurrence of the disease.

The purpose of this study is to evaluate the annual cost of patients with Wagner grade 3-4-5 DFU from the public payer's perspective in Turkey.

### MATERIALS AND METHODS

This study was conducted focused on a time frame of 1 year from the perspective of the Turkish reimbursement institution. Cost-of-illness (COI) methodology, which was developed by World Health Organization, was used in the generation of cost data<sup>[7]</sup>.

#### COI methodology

Cost is a monetary measure for the sacrifices made for achieving a certain goal. Cost is the value of a source. Economists use the concepts of "opportunity cost" or "monetary cost" in COI studies. Even though no money is spent, it is always considered that scarce resources that can be used in other areas are used. The basic idea behind cost estimation is that once a health service is provided to a person, the resources that are used will not be available anymore for other people or alternative social uses.

COI studies are used by policymakers for budget justification, determining the priorities in financing biomedical research, and development of intervention programs for preventing and treating diseases<sup>[8,9]</sup>.

Cost studies can be based on either prevalence or incidence, depending on the purpose of the analysis. The approach based on prevalence is more frequently used. In the approach based on prevalence, the total costs are calculated for a patient population in a certain area in a certain period of time<sup>[8,9]</sup>. The period of time is usually 1 year. Said studies are required for health policymakers for budget planning and decisions<sup>[8]</sup>. Studies based on



incidence calculate the lifetime cost of a patient who has a disease, starting from diagnosis to treatment, or if it is a chronic disease, until death<sup>[8,9]</sup>. The analysis perspective determines which resources will be used in the calculation<sup>[8]</sup>. Perspective shows who is affected as the resource allocation preference is made and in whose name the decisions are made<sup>[10,11]</sup>. COI analyses can be performed with different perspectives, such as societal perspective, patient perspective, or perspective of the third person/public payer<sup>[8]</sup>.

Health economy research defines the costs in two main categories. The first one is the medical costs that occur due to disease, and the second one is the other disease-associated costs including non-medical costs that occur due to disease<sup>[9-12]</sup>. There are direct and indirect costs in each category. Direct costs refer to which payments are made and indirect costs refer to which resources are lost<sup>[9]</sup>. The direct medical costs include all types of exclusive and non-exclusive uses of resources (not only monetary expenditures) such as costs related to hospital services, outpatient services, laboratory tests, supplies, prescriptions, physical therapy, care services at home and care centers, caregiver costs, and services such as ambulance, etcetera, and the use of health personnel and departments of hospitals. In addition, they include the future costs or savings such as costs of other tests with false positive or true positive results during monitoring associated with said disease and hospital admissions and treatment costs. Direct medical costs are calculated by classification according to the types of payments and expenses<sup>[10]</sup>. Indirect costs are the costs of morbidity and mortality<sup>[9]</sup>.

### Assessment and evaluation

By following a clinical path with the COI methodology, the main total expenses were reached by multiplying the number of uses of each expense item, the percentage of cases that used them, and unit costs. The direct medical costs, which are the outpatient, laboratory and imaging methods, prescribed drugs, medical supplies that are directly used during the course of treatment of disease, and the amount spent for the hospital admissions and interventions, were calculated, and non-medical direct expenses were ignored, as there were no sufficient data for Turkey. The intangible costs including pain, unhappiness, distress, misery, stress, et cetera, caused by the disease in the individual were also not taken into consideration in this study. The indirect costs including the societal costs caused by the disease, disabilities, or premature deaths were also excluded from the study.

The clinical guidelines were followed in calculating the direct costs and actual data were used for some cases.

The cost of disease was calculated by rating the Wagner classification that shows the grade of foot ulcer<sup>[13]</sup>. The Wagner classification rates of patients with DFU in Turkey were as follows: Grade 1: 7.7%, grade 2: 27.2%, grade 3: 35.2%, grade 4: 25.4%, and grade 5: 4.5%<sup>[14-16]</sup>. The costs of patients of grades 3-4-5 according to the Wagner classification were calculated.

The Medical Enforcement Declaration (MED), which is officially declared by the institution, is used for the payment of health services by the reimbursement institution in Turkey<sup>[17]</sup>. The costs for all medical services used in the calculations were obtained from MED. The drug expenses were based on the 2015 list of the Republic of Turkey Ministry of Health Turkish Medicines and Medical Devices Agency. The drugs were classified according to the active ingredients and all forms of all products included in the reimbursement list related to the active ingredients and were included in the analysis, and their average values were reflected to the calculations. Public discounts, current public paid costs, and costs related to medical materials such as all orthosis and prosthesis devices were obtained from MED. The costs were calculated according to United States dollars by using the foreign exchange rate in 2014 (\$1.00 = 1.179 TL).

The average institution cost was calculated as the admission fee for treatments administered in an outpatient clinic. The health organizations, clinical branches, and surgical branches that can administer DFU treatment were chosen, the prices of related outpatient were obtained from MED, and the average values were calculated. The average costs of pricing per admission of the patients with DFU to the outpatient clinic were included in the analysis according to said average value calculated for each branch. The cost for outpatient to which the patients were transferred for consultation was 10.12 procedure points in accordance with MED. Some of the medical materials used in payment per admission to the outpatient clinic were included in the admission fee. The examinations that were not included in the outpatient clinic admission fee were included in the costs according to MED.

Monte Carlo Simulation (MCS) was used in the study as the sensitivity analysis. MCS is a technique that uses random numbers and a trial-and-error method without using any formula. MCS provides an estimate for the statistical distribution of the possible costs. At the same time, the distribution of variables that constitute the costs is obtained. Simulation technique is a methodology employed to solve problems, not a theory. Approach of this technique to the problems varies depending on system structure and the model to be constructed based on this structure. During the simulation process, a sample is generated by the distribution of the variance observed in the proper distribution forms. Random values are used for uncertain variables. MCS can assign random values to all variables and parameters in accordance with the probabilities. The simulation is based on the random number generation. For example, for a possibility of 66 that requires osteomyelitis treatment, probability distribution according to the random numbers drawn from a normal distribution is as follows: The patient will receive the osteomyelitis treatment if the random number drawn is between 00 and 66, and for the numbers drawn between 67 and 99, the patient will be treated without a need for osteomyelitis treatment. In this study, distribution parameters were calculated at the

**Table 1** The average unit cost of outpatient to which the admissions are made in the treatment of diabetic foot ulcer

	Average unit cost (\$-PPP 2014)
Outpatient clinics	
Endocrinology and metabolic diseases	27.1
Orthopedics and traumatology	25.4
Plastic and reconstructive surgery	26.8
Dermatology	22.4
Infectious diseases	27.3
Neurology	27.5
Nephrology	27.4
Cardiovascular surgery	27.8
Physical therapy and rehabilitation	26.4
Algology	31.0
Medical ecology and hydroclimatology	25.6
Consultations	
Infectious diseases - consultation	5.2
Orthopedics and traumatology - consultation	
Cardiovascular surgery - consultation	
Plastic and reconstructive surgery - consultation	
Dermatology - consultation	

rates of diagnosis-treatment and follow-up steps for DFU. In this analysis, the distribution values were provided with the results obtained by performing 5000 simulations for each possible situation. The time horizon is 1 calendar year.

## RESULTS

The outpatient clinics visited and complications experienced by the patients according to proportional distribution vary during the treatment for DFU, and this creates different cost items in admissions made to the outpatient clinic<sup>[14,18-27]</sup>. According to the public payer's perspective, the average annual per patient outpatient cost was \$579.5 in DFU treatment (Table 1).

The distribution of imaging tests and laboratory tests that are required to be performed during the treatment of DFU was obtained from the literature. While bone curettage culture and bone biopsy were required in 66% of the patients<sup>[14,18,25-27]</sup> scintigraphy is performed in Wagner grade 4 and 5 gangrene patients (20.6%)<sup>[15,16]</sup>. Some of the laboratory tests and imaging procedures performed for the patients with DFU are included in the payment per admission made to the outpatient clinic. The items that are not included in the payment per admission were added to the calculations according to the MED list. Culture, gram staining, and antibiogram analyses must be performed in patients with DFU. In DFU treatment, the average annual per patient cost for imaging tests was \$283.2, and laboratory test cost was \$284.8 (Table 2).

Wagner 3-4-5 DFU patient groups (44.9%) are admitted to inpatient for an average of 23 d a year<sup>[18,19,21,23,26,27]</sup>. The average rate of amputation in said patients is 53.9%<sup>[14,18,19,22-24,26,27]</sup>. The average hospitalization period for the patients who are amputated is 42 d<sup>[23]</sup>. Six percent of patients are hospitalized for five days due to revascularization surgery, and the patients who have

**Table 2** Unit costs of imaging - laboratory tests used in the treatment of diabetic foot ulcer

Name of test	Average unit cost (\$-PPP 2014)
Imaging tests	
Direct foot X-ray	Included in outpatient clinic admission fee
Electrocardiogram	
Unilateral chest X-ray	
Doppler ultrasonography	32.4
Magnetic resonance angiography	55.1
Angiography	392.3
Scintigraphy	131.7
Laboratory tests	
Bone biopsy	97.6
Tissue culture	97.6
Aspiration/swab culture	33.8
Bone curettage culture	97.6
HbA1c	3.4
Bleeding profile (Pre-op)	12.2
Glucose	Included in outpatient clinic admission fee
Hemogram	
C-reactive protein	
Red blood cell sedimentation rate	
Albumin	
Kidney function tests	
Liver function tests	
Hepatitis markers	

HbA1c: Hemoglobin A1c.

graft/flap (24%) are hospitalized for an average of 12 d (Table 3)<sup>[28,29]</sup>.

The average annual per patient cost of inpatient care due to DFU was \$7357.4.

All of the patients received training on a diabetic foot. The average rate of patients receiving treatment for osteomyelitis is 66%<sup>[14,18,25-27]</sup>. Wound debridement is performed in patients at an average of 10.1%. The average rate of patients who had graft/flap was 24%. Revascularization surgery is performed in two ways: Percutaneous transluminal angioplasty (6%) or bypass (6%)<sup>[18-20,26]</sup>. An average of 8.0% of the patients receives hyperbaric oxygen treatment<sup>[18]</sup> over an average of 40 sessions<sup>[20,24]</sup>. The rate of patients receiving physical therapy and rehabilitation is 16.6% (30.8% of the amputated patients) (Table 4)<sup>[18,19,22,25,27]</sup>.

The average annual per patient cost of intervention, rehabilitation, and training for DFU was \$2291.7.

Antibiotic treatment of DFU can be grouped into three categories: Low risk, high risk, and serious risk. In wounds with low risk (24%), clindamycin (4 × 300 mg) or cephalexin (4 × 500 mg) is used for 14 d. In wounds with high risk (60.3%), the patients are admitted to the hospital and one of the following parenteral treatments is administered for 14 d: Piperacillin/tazobactam, ampicillin sulbactam, cephalexin, third generation cephalosporin + clindamycin, or ciprofloxacin + clindamycin. The patients with wounds with serious risk (15.3%) must be admitted to the hospital and one of the following treatments is administered for 14-21 d (for 6 wk if osteomyelitis exists): Ampicillin + gentamicin + clindamycin, imipenem/meropenem, vancomycin, piperacillin/clavulanate, or ticarcillin/clavulanate<sup>[30,31]</sup>.

**Table 3** Hospitalizations in departments for treatment of diabetic foot ulcer

Admission to department	Rate of patients (%)	Hospitalization period
Wound follow-up	44.9	23
Amputation surgery	53.9	42
Revascularization surgery	12.0	5
Plastic and reconstructive surgery - graft/flap	24.0	12

**Table 4** Medical and surgical interventions performed in the treatment of diabetic foot ulcer and their costs

Interventions	Rate of patients (%)	Average cost (\$-PPP 2014)
Osteomyelitis treatment	66.0	605.0
Wound debridement	10.1	813.7
Graft/flap	24.0	602.1
Percutaneous transluminal angioplasty	6.0	6250.9
Bypass	6.0	6512.4
Amputation	53.9	961.7
Hyperbaric oxygen treatment	8.0	70.0 <sup>1</sup>
Physical therapy and rehabilitation	16.6	31.3 <sup>1</sup>
Diabetic foot patient training	100.0	1.5

<sup>1</sup>Cost per session.

Insulin is used in all of the patients. Furthermore, the cost of anti-thrombotic treatment was added to the calculation for 85% of the patients.

The average annual per patient cost of medication used in the treatment of DFU was \$2545.8.

A total of 42.6% of the patients (non-ischemic wounds) use wound sheath as a medical supply<sup>[18,20,26,27]</sup>. For 53.9% of the patients, the costs of orthosis-prosthesis devices were reflected in the calculation by considering the average values for the supplies and their weighted use (Table 5).

The average annual per patient cost of medical supplies used in the treatment of DFU was \$735.0.

Some complications of methods applied in the treatment of DFU can be observed as well. During treatment of DFU, infection can be observed after amputation in 12.8% of the patients and re-amputation can be observed in 11.5% of the patients<sup>[16]</sup>. Complications such as barotraumatic otitis (10.26%) and hypoglycemia (0.85%) can be observed in patients treated with hyperbaric oxygen treatment<sup>[20]</sup>. The average cost of complications per patient with DFU was \$210.3.

The average annual per patient cost of DFU treatment in our country was \$14287.7 (Table 6).

### Sensitivity analysis

DFU includes use of some interventional procedures and pharmacological agents as well as various services provided by outpatient, inpatient, and laboratory units during diagnosis and treatment stages and also includes the cost of side effects of said procedures. Separate calculations were made for each variable for the dis-

**Table 5** Distributions of annual drug use of patients regarding drugs and other medical materials

Drugs and medical materials	Average cost (\$-PPP 2014)
Insulin	1118.9
Antibiotics - in the group of wounds with low risk	78.1
Antibiotics - in the group of wounds with moderate risk	240.0
Antibiotics - in the group of wounds with serious risk	764.5
Anti-thrombotic	1348.4
Orthosis and prosthesis devices	961.5
Wound sheath	101.8

**Table 6** The average annual cost per patient in diabetic foot treatment (\$-PPP 2014)

Cost components	Average per patient annual cost (\$-PPP)
Outpatient costs	579.5
Laboratory costs	284.8
Imaging test costs	283.2
Inpatient costs	7357.4
Intervention costs	2291.7
Drug costs	2545.8
Medical material costs	735.0
Complication costs	210.3
Total cost per patient	14287.7

tribution and accuracy of the results. Thus, the results of each variable are represented by the probabilities calculated within. As a result of the sensitivity analysis (Table 7), the standard deviation of the analysis was \$5706.6 ( $n = 5000$ ; mean = \$14146.8, 95%CI: \$13988.6-\$14304.9).

The health expenses per person are \$-PPP 1045 in 2014 in Turkey and the average annual per patient cost for DFU is 14-fold of said amount. The total health expense in 2014 in Turkey is \$-PPP 80.3 billion and the total DFU cost has a 3% share in the total annual health expenses for Turkey.

## DISCUSSION

In this study, the direct medical costs of DFU were investigated from the public payer's perspective in Turkey. In similar studies conducted on a limited number of patients and in a single center, the estimated treatment costs of DFU patients were investigated in Turkey. In a retrospective study conducted by Keskek *et al.*<sup>[21]</sup> in 2010 on patients with type 2 diabetes mellitus (T2DM) in Turkey, it was demonstrated that the costs of treatment in the hospital per patient in patients with DFU in a tertiary hospital were higher than those of the patients with T2DM without any chronic complications. The cost of one hospitalization for each patient was calculated in the study conducted by Keskek *et al.*<sup>[21]</sup>. The cost of the hospital per admission in patients with DFU was \$976.10. The cost of supplies was calculated at 42.6%, and 57.4% was calculated as cost of service. In the cost study related to DM and chronic complications conducted with 7095 patients in 2009 in Turkey, the direct costs of DFU

**Table 7** Result of sensitivity analysis for the total costs of diabetic foot ulcer

Cost components	\$-PPP (n = 5000)			
	Average	SD	95%CI	Median
Outpatient costs	576.2	196.1	568.0-584.4	565.5
Cost of imaging tests	279.4	205.6	267.9-291.0	219.2
Laboratory costs	283.3	64.7	278.7-287.7	254.8
Inpatient costs	7290.3	5047.9	6864.8-7715.8	8969.5
Intervention costs	2212.3	2347.7	1980.3-2444.4	1568.2
Drug costs	2554.4	566.3	2490.7-2618.2	2707.4
Cost of medical supplies	742.0	538.0	673.9-810.2	961.5
Cost of complications	208.7	452.7	145.0-272.5	0.0
Total cost	14146.8	5706.6	13988.6-14304.9	14615.4

were TL 1545, and in cases of amputation, the annual cost was TL 2386. In said study, the prevalence of DFU was 9.0% and its incidence was 2.0% in patients with DM, and the incidence of amputation was 0.2% and its prevalence was 1.0% in patients with DM<sup>[32]</sup>.

The costs of treatment vary according to the distribution of outpatient clinics visited by DFU patients, medication and medical materials used in treatment, laboratory and imaging tests performed, and the need for admission to a hospital and surgical intervention. The period of hospitalization is an important factor that causes high costs. The period of hospitalization is prolonged due to uncontrolled hyperglycemia, long-term wound care, infections, debridement, amputation, and newly occurring complications; therefore, the cost of treatment increases. In our study, the average direct total cost of DFU treatment per patient in our country is \$14287.7. Hospital admissions are \$7357.4 (51.5%) of said cost.

In the studies conducted based on prevalence from the perspective of the health care payer, the cost of DFU in the United States was between \$1892 and \$48354<sup>[33-36]</sup>. In the study conducted by Harrington *et al.*<sup>[33]</sup>, calculations were made using the insurance database of 1995 in the United States. The cost of DFU was \$15309. Inpatient costs are 74% of the total cost<sup>[33]</sup>. The study conducted by Stockl *et al.*<sup>[34]</sup> was performed by using the insurance database of 2000 and 2001. The cost per episode increases according to the severity of DFU. While the cost of grade 1 was \$1892 per episode, the cost of grade 4/5 was \$27721 per episode. Inpatient costs amount to 77% of the total cost<sup>[34]</sup>. In the study conducted by Sargen *et al.*<sup>[35]</sup>, the cost of DFU was studied using the insurance database of calendar year 2007. In said study, the cost of DFU was \$31363, and if amputation was performed, said cost was \$48354<sup>[35]</sup>. In the study conducted by Margolis *et al.*<sup>[36]</sup> based on the Medicare database of the United States, the amounts of reimbursement payments made for DFU and lower extremity amputations between 2006 and 2008 were calculated. The cost per patient in patients with DM with DFU was \$31600 for 2006, \$33100 for 2007, and \$35100 for 2008. The cost per patient in patients with DM who had a lower extremity amputation was \$49300, \$51200, and \$54100, respectively<sup>[36]</sup>.

Kerr *et al.*<sup>[37]</sup> calculated the cost of DFU for the National Health Service (NHS) in England in 2010-2011.

Outpatient care, inpatient care, and post-amputation care were calculated in the study conducted by Kerr *et al.*<sup>[37]</sup>. Moreover, calculations for materials such as wheelchairs, et cetera, were performed as well. In the study, it was found that 0.6% of the expenditures of NHS consisted of DFU for 2010-2011. Half of the total cost consisted of primary and community care of DFU. Some 8.8% of the total hospital costs associated with diabetes were spent for DFU. The existence of DFU increases the period of hospitalization of the patients by 2.51-fold. The outpatient cost was £4994. The inpatient cost was £3620 per admission. The post-amputation care cost was £2879 per patient.

In the study conducted by Girod *et al.*<sup>[38]</sup> in 2003 in France, the monthly cost of DFU was €697 for outpatient care and €1556 for hospital care. While 70% of the total cost consisted of hospital costs in the patients admitted to the hospital, the percentage of drug costs was 10%<sup>[38]</sup>.

Prompers *et al.*<sup>[39]</sup> prospectively calculated the societal disease cost for DFU in Europe in 2003-2004 with the approach based on incidence. In the study, in which 14 sites from 10 European countries were included, the direct cost of DFU per patient was €9446 and the cost per patient in amputated patients was €24540. The indirect cost of DFU was €645 per patient and said cost was €681 in the amputated patients. Hospital costs were 39% of the total cost of DFU<sup>[39]</sup>.

In the study conducted by Rezende *et al.*<sup>[40]</sup> in 2008 in Brazil with a simulated hypothetical cohort, approximately 30% of patients with DFU were admitted to the hospital. It was stated that extremity amputation was performed in 14% of patients with DFU. The total annual cost of patients admitted to the hospital due to DFU was approximately \$264 million (\$51 million-461 million) and said cost was \$128 million (\$24.5 million-\$222.3 million) for the amputated patients<sup>[40]</sup>.

In a study conducted in Pakistan in 2005 for investigating the direct cost of DFU treatment in a tertiary hospital, it was demonstrated that the cost of treatment increases as DFU progresses. The cost for University of Texas Classification grade 1 phase B was £21 and the same cost was £288 for grade 2 phase D and £378 for grade 3 phase D. In the study, in which 62% of the patients had a grade 2 ulcer, the average cost was £376 for major amputations and £389 for minor amputations. The average annual health expense per patient was £1.7



in Pakistan for the period the study was conducted<sup>[41]</sup>.

In a study comparing the costs of United States Medicare and private insurance patients in 2013, it was calculated that the annual treatment cost of DFU was \$11296 for Medicare (\$27040 vs \$15743) and \$15329 for privately insured (\$25931 vs \$10602) patients<sup>[42]</sup>.

In conclusion, despite the fact that it is difficult to compare the costs between countries due to the social and economic differences in terms of methods used in the treatment of DFU, said disease is a complication that decreases the quality of life of the patient, is life threatening, and significantly increases the socio-economic costs of DM.

The annual cost of DFU in Turkey was found to be similar to the results of cost studies conducted based on prevalence for the other countries.

DM-related complications are severe and will often require hospitalization for long periods. In some cases, it exposes a necessity for major surgery. The highest cost component was the hospital cost in the COI for DFU. Improvements in inpatient durations and health interventions will reduce the costs of related disease. The second leading cost component was found to be the pharmacy costs. Among these costs, antithrombotic drugs have the largest share. Increased use of generic anti-thrombotic drugs may be a powerful factor for reducing this cost.

The most effective way of reducing the costs related to DFU is the prevention of the complication itself. Another alternative is delaying the complication as long as possible. In order to prevent DFU, it will be helpful to provide training to the patients at risk and to raise awareness in patients with DM in terms of economy. Appropriate and efficient treatment of DM is a health intervention that can prevent complications. Further studies may help in discovering more effective healthcare strategies and improving the healthcare quality.

## COMMENTS

### Background

Diabetes mellitus (DM) has the highest proportion in health expenses globally. A major part of these expenses are caused by DM complications. Diabetic foot ulcer (DFU) is a frequent and severe DM complication. DFU might cause disability by going all the way to amputation. Studies have shown that DFU substantially increases mortality rates. The study has been done from Turkey Healthcare Payer's perspective. In Turkey, there are no previous studies of DFU costs done according to cost-of-illness (COI) methodology.

### Research frontiers

The current research hotspot is to identify how much DFU's cost is among all DM complications that are high in cost and which resources cost the highest among all the components of DFU costs. This way, the areas that should be intervened to lower the DFU costs will be easier to determine.

### Innovations and breakthroughs

DFU's annual mean per patient cost is \$14287.7. Hospitalization costs constitute 51.5% of these expenses. Studies done in United States and Europe report that hospitalization costs for DFU are approximately 70%. This rate is lower in Turkey. Also, hospitalization costs are a major part of DFU costs. Pharmacy costs, which are mostly anti-thrombolytic drugs and insulin treatment, constitute 18% of all

costs. In Turkey, DM constitutes 23% of all healthcare costs, and 1/6 of this is DFU expenses, which are approximately 3% of all health care expenses in Turkey.

## Applications

With this study, it has been shown that DFU constitutes 3% of all health care costs in Turkey. Shortening the time spent hospitalized and improving the interventions done in hospitals should lower the costs substantially. Using generic anti-thrombolytic agents and manufacturing insulin locally in Turkey are powerful moves that might decrease these costs. The study, which is done by COI methodology, will supply the convenient data needed to compare the costs between Turkey and other countries.

## Terminology

Cost in health economics refers to the resources consumed during the provision of health care. COI study aims to determine the total economic impact of a disease or health condition on society through the identification, measurement, and valuation of all direct and indirect costs. Sensitivity analysis is a way to analyze the impact of uncertainty on an economic analysis or a decision. Simulation is a modeling technique that makes it possible to observe the causation in the system and the actions of the real system under different circumstances. Monte Carlo Simulation (MCS) is a technique that uses random numbers and a trial-and-error method without using any formula. MCS provides an estimate for the statistical distribution of the possible costs.

## Peer-review

In the present study, the authors evaluate the annual cost of patients with diabetic foot ulcers in Turkey. In general, the manuscript is well written, straightforward and very descriptive.

## REFERENCES

- 1 **International Diabetes Federation.** IDF Diabetes Atlas. 6th ed. Basel, Switzerland: International Diabetes Federation, 2013: 11-16
- 2 **Public Health Institution of Turkey.** National Diabetes Programme 2015-2020. Ankara, Turkey: Republic of Turkey Ministry of Health, 2014: 25-38
- 3 **Singh N, Armstrong DG, Lipsky BA.** Preventing foot ulcers in patients with diabetes. *JAMA* 2005; **293**: 217-228 [PMID: 15644549 DOI: 10.1001/jama.293.2.217]
- 4 **Reiber GE, Vileikyte L, Boyko EJ, del Aguila M, Smith DG, Lavery LA, Boulton AJ.** Causal pathways for incident lower-extremity ulcers in patients with diabetes from two settings. *Diabetes Care* 1999; **22**: 157-162 [PMID: 10333919]
- 5 **Ollendorf DA, Kotsanos JG, Wishner WJ, Friedman M, Cooper T, Bittoni M, Oster G.** Potential economic benefits of lower-extremity amputation prevention strategies in diabetes. *Diabetes Care* 1998; **21**: 1240-1245 [PMID: 9702427 DOI: 10.2337/diacare.21.8.1240]
- 6 **Ministry of Health and Turkish Diabetes Foundation.** Diabetes 2020: Vision and Targets - Turkey Final Report. Ankara, Turkey: Turkish Diabetes Foundation, 2010: 57-60
- 7 **Cowley P, Bodabilla L, Musgrove P, Saxenian H.** Content and Financing of an Essential National Package of Health Services, Global Assessments in the Health Sector. World Health Organization, 1994: 171-181
- 8 **Kobelt G.** Forms of Health Economic Evaluation. In: Kobelt G. Health economics: An introduction to economic evaluation. 3rd ed. London: Office of Health Economics, 2013: 12-31
- 9 **Rice DP.** Cost-of-illness studies: fact or fiction? *Lancet* 1994; **344**: 1519-1520 [PMID: 7983947 DOI: 10.1016/S0140-6736(94)90342-5]
- 10 **Rice DP.** Cost of illness studies: what is good about them? *Inj Prev* 2000; **6**: 177-179 [PMID: 11003181 DOI: 10.1136/ip.6.3.177]
- 11 **Russell LB, Siegel JE, Daniels N, Gold MR, Luce BR, Mandelblatt JS.** Cost-effectiveness analysis as a Guide to resource allocation in Health: Roles and limitations. In: Gold MR, Siegel JE, Russell LB, Weinstein MC, editors. Cost-effectiveness in health and medicine. New York: Oxford University Press, 1996: 3-24
- 12 **Luce BR, Manning WG, Lipscomb J.** Estimating costs in Cost-

- effectiveness analysis. In: Gold MR, Siegel JE, Russell LB, Weinstein MC, editors. Cost-effectiveness in health and medicine. New York: Oxford University Press, 1996: 176-213
- 13 **Wagner FW.** The dysvascular foot: a system for diagnosis and treatment. *Foot Ankle* 1981; **2**: 64-122 [PMID: 7319435 DOI: 10.1177/107110078100200202]
  - 14 **Yesil S, Akinci B, Yener S, Bayraktar F, Karabay O, Havitcioglu H, Yapar N, Atabey A, Kucukyavas Y, Comlekci A, Eraslan S.** Predictors of amputation in diabetics with foot ulcer: single center experience in a large Turkish cohort. *Hormones* (Athens) 2009; **8**: 286-295 [PMID: 20045802 DOI: 10.14310/horm.2002.1245]
  - 15 **Demir T, Akinci B, Yesil S.** Diagnosis and treatment of diabetic foot ulcers. *Dokuz Eylul Medical Journal* 2007; **21**: 63-70
  - 16 **Arikan Y, Kuzgun U, Sever C, Armagan R.** The role of orthopedic's in multidisciplinary approach to diabetic foot wounds. *The Medical Bulletin of Sisli Etfal Hospital* 2010; **44**: 106-112
  - 17 **Republic of Turkey Social Security Institution.** The Medical Enforcement Declaration. 2014
  - 18 **Altindas M, Kilic A, Cinar C, Bingol UA, Ozturk G.** The epidemiology of foot wounds in patients with diabetes: a description of 600 consecutive patients in Turkey. *J Foot Ankle Surg* 2011; **50**: 146-152 [PMID: 21353997 DOI: 10.1053/j.jfas.2010.12.017]
  - 19 **Tabur S, Eren MA, Çelik Y, Dağ OF, Sabuncu T, Sayiner ZA, Savas E.** The major predictors of amputation and length of stay in diabetic patients with acute foot ulceration. *Wien Klin Wochenschr* 2015; **127**: 45-50 [PMID: 25398288 DOI: 10.1007/s00508-014-0630-5]
  - 20 **Akgül EA, Karakaya J, Aydin S.** Role of comorbidities as limiting factors to the effect of hyperbaric oxygen in diabetic foot patients: a retrospective analysis. *Diabetes Ther* 2014; **5**: 535-544 [PMID: 25273365]
  - 21 **Keskek SO, Kirim S, Yanmaz N.** Estimated costs of the treatment of diabetic foot ulcers in a tertiary hospital in Turkey. *Pak J Med Sci* 2014; **30**: 968-971 [PMID: 25225508 DOI: 10.12669/pjms.305.5182]
  - 22 **Karakas A, Arslan E, Cakmak T, Aydin I, Akgul EO, Demirbas S.** Predictive Value of Soluble CD14, Interleukin-6 and Procalcitonin For Lower Extremity Amputation in People with Diabetes with Foot Ulcers: A Pilot Study. *Pak J Med Sci* 2014; **30**: 578-582 [PMID: 24948983 DOI: 10.12669/pjms.303.4575]
  - 23 **Mutluoglu M, Sivrioglu AK, Eroglu M, Uzun G, Turhan V, Ay H, Lipsky BA.** The implications of the presence of osteomyelitis on outcomes of infected diabetic foot wounds. *Scand J Infect Dis* 2013; **45**: 497-503 [PMID: 23384323 DOI: 10.3109/00365548.2013.765589]
  - 24 **Aydin F, Kaya A, Karapinar L, Kumbaraci M, Imerci A, Karapinar H, Karakuzu C, Incesu M.** IGF-1 Increases with Hyperbaric Oxygen Therapy and Promotes Wound Healing in Diabetic Foot Ulcers. *J Diabetes Res* 2013; **2013**: 567834 [PMID: 23671876 DOI: 10.1155/2013/567834]
  - 25 **Korkmaz M, Erdoğan Y, Balci M, Senarslan DA, Yılmaz N.** Preoperative medical treatment in patients undergoing diabetic foot surgery with a Wagner Grade-3 or higher ulcer: a retrospective analysis of 52 patients. *Diabet Foot Ankle* 2012; **3** [PMID: 22919456 DOI: 10.3402/dfa.v3i0.18838]
  - 26 **Altay FA, Sencan İ, Şentürk GÇ, Altay M, Güvenman S, Ünverdi S, Açıkgöz ZC.** Does treatment affect the levels of serum interleukin-6, interleukin-8 and procalcitonin in diabetic foot infection? A pilot study. *J Diabetes Complications* 2012; **26**: 214-218 [PMID: 22521320 DOI: 10.1016/j.jdiacomp.2012.03.018]
  - 27 **Ertugrul BM, Oncul O, Tulek N, Willke A, Sacar S, Tunccan OG, Yilmaz E, Kaya O, Ozturk B, Turhan O, Yapar N, Ture M, Akin F.** A prospective, multi-center study: factors related to the management of diabetic foot infections. *Eur J Clin Microbiol Infect Dis* 2012; **31**: 2345-2352 [PMID: 22354524 DOI: 10.1007/s10096-012-1574-1]
  - 28 **McCartan B, Dinh T.** The use of split-thickness skin grafts on diabetic foot ulcerations: a literature review. *Plast Surg Int* 2012; **2012**: 715273 [PMID: 22666573 DOI: 10.1155/2012/715273]
  - 29 **Puttirutvong P.** Meshed skin graft versus split thickness skin graft in diabetic ulcer coverage. *J Med Assoc Thai* 2004; **87**: 66-72 [PMID: 14971537]
  - 30 **Yaman E.** As Scintigraphic Determination of the Amputation Levels on the Diabetic Foot. Isparta, Turkey: Süleyman Demirel University, 2009
  - 31 **Lipsky BA, Berendt AR, Cornia PB, Pile JC, Peters EJ, Armstrong DG, Deery HG, Embil JM, Joseph WS, Karchmer AW, Pinzur MS, Senneville E.** 2012 Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. *Clin Infect Dis* 2012; **54**: e132-e173 [PMID: 22619242 DOI: 10.1093/cid/cis346]
  - 32 **Malhan S, Oksuz E, Babineaux SM, Ertekin A, Palmer JP.** Assessment of the Direct Medical Costs of Type 2 Diabetes Mellitus and its Complications in Turkey. *Turk Jem* 2014; **2**: 39-43 [DOI: 10.4274/tjem.2441]
  - 33 **Harrington C, Zagari MJ, Corea J, Klitenic J.** A cost analysis of diabetic lower-extremity ulcers. *Diabetes Care* 2000; **23**: 1333-1338 [PMID: 10977028 DOI: 10.2337/diacare.23.9.1333]
  - 34 **Stockl K, Vanderplas A, Tafesse E, Chang E.** Costs of lower-extremity ulcers among patients with diabetes. *Diabetes Care* 2004; **27**: 2129-2134 [PMID: 15333473 DOI: 10.2337/diacare.27.9.2129]
  - 35 **Sargen MR, Hoffstad O, Margolis DJ.** Geographic variation in Medicare spending and mortality for diabetic patients with foot ulcers and amputations. *J Diabetes Complications* 2013; **27**: 128-133 [PMID: 23062327 DOI: 10.1016/j.jdiacomp.2012.09.003]
  - 36 **Margolis DJ, Malay DS, Hoffstad OJ, Leonard CE, MacCurdy T, Tan Y, Molina T, de Nava KL, Siegel KL.** Economic burden of diabetic foot ulcers and amputations. Diabetic Foot Ulcers. Data Points 3 (prepared by the University of Pennsylvania DECIDE Center, under Contract No. HHS290200500411). Rockville, MD: Agency for Healthcare Research and Quality, 2011
  - 37 **Kerr M, Rayman G, Jeffcoate WJ.** Cost of diabetic foot disease to the National Health Service in England. *Diabet Med* 2014; **31**: 1498-1504 [PMID: 24984759 DOI: 10.1111/dme.12545]
  - 38 **Girod I, Valensi P, Laforêt C, Moreau-Defarges T, Guillon P, Baron F.** An economic evaluation of the cost of diabetic foot ulcers: results of a retrospective study on 239 patients. *Diabetes Metab* 2003; **29**: 269-277 [PMID: 12909815]
  - 39 **Prompers L, Huijberts M, Schaper N, Apelqvist J, Bakker K, Edmonds M, Holstein P, Jude E, Jirkovska A, Mauricio D, Piaggese A, Reike H, Spraul M, Van Acker K, Van Baal S, Van Merode F, Uccioli L, Urbancic V, Ragnarson Tennvall G.** Resource utilisation and costs associated with the treatment of diabetic foot ulcers. Prospective data from the Eurodiale Study. *Diabetologia* 2008; **51**: 1826-1834 [PMID: 18648766 DOI: 10.1007/s00125-008-1089-6]
  - 40 **Rezende KF, Ferraz MB, Malerbi DA, Melo NH, Nunes MP, Pedrosa HC, Chacra AR.** Predicted annual costs for inpatients with diabetes and foot ulcers in a developing country-a simulation of the current situation in Brazil. *Diabet Med* 2010; **27**: 109-112 [PMID: 20121897 DOI: 10.1111/j.1464-5491.2009.02871.x]
  - 41 **Ali SM, Fareed A, Humail SM, Basit A, Ahmedani MY, Fawwad A, Miyan Z.** The personal cost of diabetic foot disease in the developing world--a study from Pakistan. *Diabet Med* 2008; **25**: 1231-1233 [PMID: 19046203 DOI: 10.1111/j.1464-5491.2008.02529.x]
  - 42 **Rice JB, Desai U, Cummings AK, Bimbaum HG, Skomicki M, Parsons N.** Medical, Drug, and Work-Loss Costs of Diabetic Foot Ulcers. *Value in Health* 2013; **16**: A12 [DOI: 10.1016/j.jval.2013.03.075]

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## Observational Study

# Predictors of hypoglycemia in insulin-treated patients with type 2 diabetes mellitus in Basrah

Dhuha Tarik Nassar, Omran S Habib, Abbas Ali Mansour

Dhuha Tarik Nassar, Basrah Health Directorate, Basrah 61013, Iraq

Omran S Habib, Department of Epidemiology, Basrah College of Medicine, Basrah 61013, Iraq

Abbas Ali Mansour, Al-Faiha Specialized Diabetes, Endocrine and Metabolism Center (FDEMC), Chair Diabetes, Endocrine and Metabolism Division, Department of Medicine, Basrah College of Medicine, Basrah 61013, Iraq

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**Correspondence to:** Abbas Ali Mansour, Professor of Medicine, Consultant Endocrinologist, Al-Faiha Specialized Diabetes, Endocrine and Metabolism Center (FDEMC), Chair Diabetes, Endocrine and Metabolism Division, Department of Medicine, Basrah College of Medicine, Hattin Post Office, P.O Box: 142, Basrah 61013, Iraq. [aambaam@gmail.com](mailto:aambaam@gmail.com)  
 Telephone: +964-780-1403706

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## Abstract

### AIM

To measure the incidence and determinants (predictors) of hypoglycemia among patients with type 2 diabetes mellitus (T2DM) who were on insulin treatment for at least one year.

### METHODS

The present study is an out-patients based inquiry about the risk and predictors of hypoglycemia among patients with T2DM seeking care at the Al-Faiha Specialized Diabetes, Endocrine, and Metabolism Center, in Basrah over a period of 7 mo (from 15<sup>th</sup> of April, 2013 to 15<sup>th</sup> of October, 2013). The data used in the study were based on all detailed interview and selected laboratory investigations. A total of 336 patients could be included in the study.

### RESULTS

The incidence of overall hypoglycemia among the studied patients was 75.3% within the last 3 mo preceding the interview. The incidence of hypoglycemia subtypes were 10.2% for severe hypoglycemia requiring medical assistance in the hospital, 44.36% for severe hypoglycemia treated at home by family; this includes both confirmed severe hypoglycemia with an incidence rate of 14.6% and unconfirmed severe hypoglycemia for which incidence rate was 29.76%. Regarding mild self-treated hypoglycemia, the incidence of confirmed mild hypoglycemia was 21.42%, for unconfirmed mild

hypoglycemia the incidence rate was 50.0% and for total mild hypoglycemia, the incidence rate was 71.42%. The most important predictors of hypoglycemia were a peripheral residence, increasing knowledge of hypoglycemia symptoms, in availability and increasing frequency of self-monitoring blood glucose, the presence of peripheral neuropathy, higher diastolic blood pressure, and lower Hemoglobin A1c.

### CONCLUSION

Hypoglycemia is very common among insulin-treated patients with T2DM in Basrah. It was possible to identify some important predictors of hypoglycemia.

**Key words:** Diabetes mellitus; Insulin; Hypoglycemia; Out-patient; Type 2

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**Core tip:** Outpatients study aimed to assess the frequency of hypoglycemia and their predictors among patients with type 2 diabetes mellitus on insulin for at least one year. The majority of patients (75.3%) had hypoglycemia in the preceding 3 mo. We identify some important predictors of hypoglycemia.

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## INTRODUCTION

Hypoglycemia is very frequent and serious complication of insulin therapy, especially in those with intensive treatment and unawareness of hypoglycemia is a very dangerous situation that complicated the problem more<sup>[1]</sup>.

Severe hypoglycemia is defined to be an episode of hypoglycemia in which a patient requires help from another people. Thus, patients who are more compliant or precise in using their medication to lower their glucose levels are at greatest risk of hypoglycemia and its sequels<sup>[2,3]</sup>.

Confirmed symptomatic hypoglycemia is an event during which classic symptoms of hypoglycemia was confirmed simultaneously by measured plasma glucose concentration < 70 mg/dL (3.9 mmol/L)<sup>[3]</sup>. Asymptomatic hypoglycemia is an attack not accompanied by classic symptoms of hypoglycemia but with a measured plasma glucose concentration < 70 mg/dL (3.9 mmol/L). Furthermore, probable symptomatic hypoglycemia is defined as symptoms of hypoglycemia that not proven by measuring simultaneous plasma glucose and assumed to be due to a plasma glucose concentration < 70 mg/dL

(3.9 mmol/L).

It's well known that people with diabetes most of the times treat symptoms of hypoglycemia with the diet without measuring their plasma glucose at the same time. That why these episodes can be considered as probable hypoglycemia. These unconfirmed hypoglycemic episodes reported by the patients may affect the results of studies intended to evaluate the drugs that affect plasma glucose, but they should be declared by any mean as self-reported hypoglycemic episodes that are not confirmed.

Finlay, we have to define relative hypoglycemia. These symptoms of hypoglycemia reported by patients with diabetes but associated with simultaneously measured plasma glucose concentration > 70 mg/dL (3.9 mmol/L).

This last group of hypoglycemic episodes is seen more in those with long-standing diabetes with poor control. They per say may not be harmful, and they are no suitable outcome measures in clinical studies needed to evaluate drug therapy in diabetes, but again have to be reported though the symptoms happen with plasma glucose levels > 70 mg/dL (3.9 mmol/L).

In this study, we assess the frequency of hypoglycemia among insulin-treated patients with type 2 diabetes mellitus (T2DM) who were on insulin for at least one year.

## MATERIALS AND METHODS

### Study design

The study is a cross-sectional study investigating retrospectively the experience of hypoglycemia among patients with type T2DM receiving insulin for at least one year preceding the time of study who attended Al-Faiha Specialized Diabetes, Endocrine, and Metabolism Center (FDEMC). The study extended in the data collection phase over five month period from 15<sup>th</sup> of April to 15<sup>th</sup> of October 2013.

### Sampling methods and sample size

A total of 336 patients were enrolled in the study. Data were collected through direct interview with the patients after ensuring their verbal agreement to take part in the study. On average 5-6 patients could be fully interviewed each working day.

### Inclusion criteria

All patients with T2DM (no age limit) including men and non-pregnant women receiving insulin for at least one year proceeding the time of study who attended FDEMC. Informed verbal consent was taken from all patients, and the ethical committee of Basrah College of Medicine approved the study.

### Questionnaire and data collection

A special questionnaire form was prepared for the purpose of data collection for this study. It covered the following



aspects. Personal characteristics including information on name, age, sex, job, address, level of education, marital status. Medical characteristics including family history of diabetes mellitus, duration of diabetes, duration of insulin use, type of insulin use, frequency of insulin used per day, dose of insulin per time of administration per day, total dose of insulin per day (for the preceding 3 mo), whether the patient is on oral hypoglycemic drug, its type dose, and frequency. Other questions include who inject insulin to the patient, state of patient's vision, patient's mobility, the source of medication, knowledge of the patient about symptoms of hypoglycemia.

Information on hypoglycemic attacks, including whether the patient had hypoglycemic attacks during the preceding three before the interview, type of hypoglycemic attack and timing during the day, events precipitating hypoglycemia, whether self-monitoring blood glucose (SMBG) device was available and the frequency of its use, did hypoglycemic attack was confirmed by SMBG or by venous blood and what was the blood glucose level, awareness of the patient for hypoglycemia.

History of other co-morbidities such as hypertension (HTN), ischemic heart disease (IHD), cerebrovascular accidents (CVA), amputation, chronic kidney disease (CKD), diabetic foot, and peripheral neuropathy (PNP).

The use of insulin by another family member at home or outside the home and whether the patient takes other concomitant medications with the insulin. Measurement of height and weight to obtain a body mass index (BMI) (done by a nurse on the day of the visit). Investigations were done in the laboratory of FDEMC on the day of the visit, and these include measurement of glycated hemoglobin (HbA1C), serum creatinine, and urine for albumin.

### Definition of variables

**Details related to hypoglycemia:** Respondent's knowledge of hypoglycemia symptoms was grouped into yes or no. An incident of hypoglycemia, the respondent was asked if he or she developed, at least, one episode of hypoglycemia during the last 3 mo; this includes asking about the symptoms of hypoglycemia, and the answer was grouped into yes or no.

**Type of hypoglycemia:** By adopting the ADA definition of hypoglycemia<sup>[3,4]</sup>, it was classified into: (1) severe need third party help in the hospital by a doctor; severe need second party help at home by family. Severe hypoglycemia also subdivided into confirmed severe hypoglycemia, and unconfirmed severe hypoglycemia; and (2) mild self-treated hypoglycemia was also subdivided into confirmed mild hypoglycemia and unconfirmed mild hypoglycemia. Confirmation of hypoglycemia (what was blood glucose level at the time of the attack?) was grouped into: By SMBG, by venous blood or not (hypoglycemia not confirmed). Awareness of hypoglycemia was grouped into yes or no<sup>[1]</sup>.

### Statistical analysis

Data were coded according to the variable definition and entered into a computer program: Statistical Package for Social Science (SPSS - version 20). Data were analyzed and presented in suitable tables. Three layers of tables are presented: Descriptive tables describing patients socio-demographic and medical characteristics, Cross-tabulations of the history of hypoglycemia with probable risk factors.  $\chi^2$  or Fisher's Exact test was used to find out the statistical association, *P* value < 0.05 was considered significant. Logistic regression analysis was done to identify significant predictors of hypoglycemia.

## RESULTS

Socio-demographic characteristics of the studied patients included age range was 29-88 years with mean age of 54.47 years; 38.1% were in the age group 50-59 year; 28.0% were in the age group 60-69 year. Regarding gender, female cases showed predominance forming 61.9% compared to males who accounted for 38.1% of cases. More than one-quarter of patients (29.8%) had completed primary schooling. The majority were married accounting for 80.1%. Regarding residence, most of the respondents lived in Basrah city (67.3%).

Some medical aspects of the studied patients, where 40.2% have more than one 1<sup>st</sup> and 2<sup>nd</sup> degree relative with DM, regarding the frequency of insulin administration/day; 44.6% of patients received insulin three times daily, 42.9% received insulin twice daily.

On co-morbidities, 74.4% of them had HTN, 17.3%, and CVA reported IHD was reported by 6%. Amputation was evident in 3%, CKD in 26.8% and diabetic foot in 27.1%, and PNP in 90.2%.

Most of the patients (75.6%) injected themselves insulin and needed no external support, about vision; 81% of patients reported good vision, 87.8% were mobile alone without assistance. The majority of patients (66.1%) received insulin from more than one source. Regarding knowledge of hypoglycemia symptoms; 95.2% reported that they knew hypoglycemia symptoms.

Table 1 shows the incidence (%) of hypoglycemia (total and subtypes) in the last 3 mo as reported by the patients. The majority of patients (75.3%) had hypoglycemia in the preceding 3 mo. The incidence of hypoglycemia subtypes was 10.2% for severe hypoglycemia requiring medical assistance in the hospital, 44.36% for severe hypoglycemia treated at home by family; this includes both confirmed severe hypoglycemia with an incidence rate of 14.6% and unconfirmed severe hypoglycemia for which incidence rate was 29.76%.

Regarding mild self-treated hypoglycemia, the incidence of confirmed mild hypoglycemia was 21.42%, for unconfirmed mild hypoglycemia the incidence rate was 50.0% and for total mild hypoglycemia, the incidence rate was 71.42%.

More than half of the patients who had experienced hypoglycemia during the preceding 3 mo (57.6%) had

**Table 1** Incidence, types, timing, and causes of hypoglycemia in 336 patients

Variable	n (%)
Hypoglycemia in the last 3 mo	253 (75.3)
Type of hypoglycemia	
Severe treated in hospital	34 (10.2)
Sever confirmed hypoglycemia treated at home by family ( $\leq 70$ mg/dL)	49 (14.6)
Severe unconfirmed hypoglycemia treated at home by family or blood glucose $> 70$ mg/dL	100 (29.7)
Mild confirmed hypoglycemia ( $\leq 70$ mg/dL)	72 (21.4)
Mild unconfirmed hypoglycemia or blood glucose $> 70$ mg/dL	168 (50.0)
Total severe hypoglycemia treated at home by family	149 (44.3)
Total mild hypoglycemia	240 (71.4)
Timing of hypoglycemia in the last 3 mo	
Nocturnal	22 (8.7)
Day time	83 (32.8)
Nocturnal and day time	148 (58.5)
Precipitating factors hypoglycemia	
Missed meal, delayed meal, eating a less amount of food	214 (84.6)
Performing an exercise	42 (16.6)
Doctor change the dose of insulin recently	12 (4.7)
Insulin dose adjusted by the patient, errors in the dose of insulin	7 (2.8)
No obvious cause	22 (8.7)
Awareness of hypoglycemia in the last 3 mo	
No	19 (7.5)

developed both nocturnal and daytime hypoglycemia.

The most common causes of hypoglycemia are factors related to a meal including missed meal, delayed meal or eating a less amount of food, and the majority of the patients are aware of hypoglycemia symptoms in the preceding 3 mo.

#### **Determinants of hypoglycemia during the preceding 3 mo**

In Table 2, although a higher percentage of hypoglycemia was reported in the younger age group 29-39 year and among females; there is no significant association between age and gender with experience of hypoglycemia during the preceding 3 mo;  $P > 0.05$ . There is a highly significant association with the education of respondents;  $P = 0.016$  with the highest percentage in those who had completed primary schooling. There is no significant association between marital status and residence with experience of hypoglycemia during the preceding 3 mo ( $P > 0.05$ ).

Table 3 shows there is no significant association of DM family history, duration of DM and duration of insulin treatment with experience of hypoglycemia during last 3 mo;  $P > 0.05$ . No significant association between type of insulin and experience of hypoglycemia during the preceding 3 mo ( $P > 0.05$ ); while there is a highly significant association between the frequency of insulin administration per day and total dose of insulin per day with hypoglycemia in the last 3 mo ( $P < 0.05$ ).

In Table 4, there is no significant association between dose of regular, premix and Neutral Protamine Hagedorn (NPH) insulin and experience of hypoglycemia during last 3 mo ( $P > 0.05$ ). There is no significant association

**Table 2** Relation of hypoglycemia in the last 3 mo with age, gender, education, marital status and residence among 336 patients

	Hypoglycemia in the last 3 mo		Total (n)	P value
	Yes	No		
Age (yr)	n (%)	n (%)		
29-39	22 (81.5)	5 (18.5)	27	0.944
40-49	48 (73.8)	17 (26.2)	65	
50-59	97 (75.8)	31 (24.2)	128	
60-69	70 (74.5)	24 (25.5)	94	
$\geq 70$	16 (72.7)	6 (27.3)	22	
Gender				
Male	93 (72.7)	35 (27.3)	128	0.776
Female	160 (76.9)	48 (23.1)	208	
Education				
Illiterate	61 (76.2)	19 (23.8)	80	0.016
Just literate	37 (75.5)	12 (24.5)	49	
Primary school	83 (83.0)	17 (17.0)	100	
Intermediate school	44 (77.2)	13 (22.8)	57	
Secondary school	8 (50.0)	8 (50.0)	16	
College and more	20 (58.8)	14 (41.2)	34	
Marital status				
Single	5 (83.3)	1 (16.7)	6	0.604
Married	200 (74.3)	69 (25.7)	269	
Divorced	3 (60.0)	2 (40.0)	5	
Widowed	45 (80.4)	11 (19.6)	56	
Residence				
Basrah city	164 (72.6)	62 (27.4)	226	0.215
Northern Basrah	40 (72.4)	13 (27.65)	53	
Southern Basrah	5 (83.3)	1 (16.7)	6	
Eastern Basrah	12 (100.0)	0 (0.0)	12	
Western Basrah	32 (82.1)	7 (17.9)	39	

between family support, vision, mobility and source of medications with experience of hypoglycemia during the preceding 3 mo;  $P > 0.05$ . While there was a significant association between knowledge of hypoglycemia symptoms and experience of hypoglycemia ( $P < 0.05$ ).

Table 5 shows there is no significant association regarding availability and frequency of SMBG with experience of hypoglycemia during last 3 mo ( $P > 0.05$ ).

Also, there is no significant association between HTN, CVA, CKD, amputation and diabetic foot with experience of hypoglycemia during the preceding 3 mo ( $P > 0.05$ ), but a significant association does exist between IHD and PNP with experience of hypoglycemia during the preceding 3 mo ( $P < 0.05$ ).

There is no significant association between insulin use by other family members, concomitant use of other medication and type of medication with experience of hypoglycemia during the preceding 3 mo ( $P > 0.05$ ).

Table 6 shows that there is no significant association between BMI; systolic blood pressure; diastolic blood pressure; HbA1c; serum creatinine; urine for albumin with experience of hypoglycemia during last 3 mo ( $P > 0.05$ ).

#### **Logistic regression analysis**

To overcome some of the interaction and confounding effects of the various predictors used in this study; a logistic regression analysis was done. Experience of hypoglycemia in the last 3 mo was used as the dependent

**Table 3** Relation of hypoglycemia in the last 3 mo with diabetes mellitus family history, duration of diabetes mellitus, duration of insulin treatment, type of insulin, frequency and total dose

	Hypoglycemia in the last 3 mo		Total (n)	P value
	Yes n (%)	No n (%)		
Family history of DM				
None	85 (78.7)	23 (21.3)	108	0.601
One	68 (73.1)	25 (26.9)	93	
More than one	100 (74.1)	35 (25.9)	135	
Duration of DM (yr)				
1-10	140 (75.7)	45 (24.3)	185	0.877
11-20	92 (76.0)	29 (24.0)	121	
21-30	18 (69.2)	8 (30.8)	26	
≥ 31	3 (75.0)	1 (25.0)	4	
Duration of insulin treatment (yr)				
1-10	238 (74.8)	80 (25.2)	318	0.578
> 10	15 (83.3)	3 (16.7)	18	
Type of insulin				
Premix	75 (72.1)	29 (27.9)	104	0.239
Regular	6 (75.0)	2 (25.0)	8	
NPH	24 (64.9)	13 (35.1)	37	
Combination of 2 or 3 insulin types	148 (79.1)	39 (20.9)	187	
Frequency of insulin administration/d				
Once	3 (30.0)	7 (70.0)	10	0.001
Twice	107 (74.3)	37 (25.7)	144	
Thrice	126 (84.0)	24 (16.0)	150	
≥ Four times	17 (53.1)	15 (46.9)	32	
Total dose of insulin (unit/d)				
< 20	3 (37.5)	5 (62.5)	8	0.007
21-40	46 (75.4)	15 (24.6)	61	
41-60	121 (79.1)	32 (20.9)	153	
61-80	59 (81.9)	13 (18.1)	72	
81-100	17 (56.7)	13 (43.3)	30	
> 100	7 (58.3)	5 (41.7)	12	

DM: Diabetes mellitus; NPH: Neutral protamine hagedorn.

outcome variable, only peripheral residence, knowledge of hypoglycemia symptoms, availability and increasing frequency of SMBG, presence of PNP, higher diastolic blood pressure, and lower HbA1c were significant and independent predictors. All other studied variables were not predictors (Table 7).

## DISCUSSION

The results of this study showed that most of the studied patients had experienced at least one episode of hypoglycemia during the last 3 mo (75.3%). The reported risk of hypoglycemia in this study is higher than the 43.3% that was reported by Fritsche *et al.*<sup>[5]</sup>, 45% by Donnelly *et al.*<sup>[6]</sup> and the 64% by Henderson *et al.*<sup>[7]</sup>.

Although it is agreed that patients remember major events such as major hypoglycemia requiring second party help by medical personnel or by family easier than minor self-treated events; in the present study patients seemed to recall both minor and major hypoglycemic episodes including those hypoglycemic episodes which were treated in hospital or at home by family; this can be explained by the fact that hypoglycemic events including minor ones cause stress, anxiety and other sympathoadrenal symptoms that can be remembered even if it happened several mo ago especially if they are

frequent<sup>[8,9]</sup>.

Incidence rates of hypoglycemia subtypes (severe and mild) in the present study were generally higher than that reported in other studies. By Donnelly *et al.*<sup>[6]</sup> the incidence of severe hypoglycemia requiring assistance was 3%, by Henderson *et al.*<sup>[7]</sup> it was 15% and by United Kingdom Hypoglycemia Study Group it was 7% (incidence of mild hypoglycemia 51%)<sup>[10]</sup>. This excess in incidence may be due to poor adherence to the prescribed treatment regimens, fluctuation in the timing of meals and insulin doses, low education, presence of other diabetes complications especially diabetic nephropathy and autonomic neuropathy. Some patients who experienced minor hypoglycemia may receive unnecessary help from their relatives or unnecessary treatment in the emergency room; this could have lead to overestimation of severe hypoglycemia.

The incidence of severe hypoglycemia treated at home by the family and was confirmed by blood glucose measurement was lower than the incidence of severe unconfirmed hypoglycemia (14.6% vs 29.76%) and the same thing for mild self-treated hypoglycemia (incidence of confirmed hypoglycemia was 21.42% vs 50.0% for mild unconfirmed ones), this might be due to many patients choose to treat hypoglycemia without measuring blood glucose by SMBG or it is unavailable

**Table 4** Relation of hypoglycemia during the last 3 mo with dose of insulin, family/social support, mobility, source of medications and knowledge of hypoglycemia symptoms

	Hypoglycemia in the last 3 mo		Total (n)	P value
	Yes n (%)	No n (%)		
Regular dose (unit)				
1-10	7 (100.0)	0 (0.0)	7	0.347
11-20	84 (80.2)	21 (19.8)	105	
21-30	57 (77.0)	17 (23.0)	74	
> 30	5 (62.5)	3 (37.5)	8	
Total	153 (79.0)	41 (21.0)	194	
Premix dose (unit)				
1-10	2 (100.0)	0 (0.0)	2	0.45
11-20	78 (78.8)	21 (21.2)	99	
21-30	86 (77.3)	25 (22.7)	111	
> 30	10 (62.5)	6 (37.5)	16	
Total	176 (77.1)	52 (22.9)	228	
NPH dose (unit)				
1-10	4 (100.0)	0 (0.0)	4	0.528
11-20	37 (73.1)	14 (26.9)	51	
21-30	27 (66.7)	14 (33.3)	41	
> 30	6 (85.7)	1 (14.3)	7	
Total	74 (72.4)	29 (27.6)	103	
Family/social support				
Self	190 (74.8)	64 (25.2)	254	0.914
Others	51 (76.1)	16 (23.9)	67	
Self and others	12 (80.0)	3 (20.0)	15	
Vision				
Good	203 (74.6)	69 (25.4)	272	0.560
Poor	50 (78.1)	14 (21.9)	64	
Mobility				
Mobile alone	223 (75.6)	72 (24.4)	295	0.698
Mobile with assistance or use wheel chair	9 (81.8)	2 (18.2)	11	
Walk on stick	21 (70.0)	9 (30.0)	30	
Source of medications				
FDEMC <sup>1</sup>	63 (75.0)	21 (25.0)	84	0.507
Public clinic	7 (58.3)	5 (41.7)	12	
Private sector	13 (72.2)	5 (27.8)	18	
More than one source	170 (76.6)	52 (23.4)	222	
Knowledge of hypoglycemia symptoms				
Yes	246 (76.9)	74 (23.1)	320	0.003

<sup>1</sup>Al-Faiha Specialized Diabetes, Endocrine and Metabolism Center. NPH: Neutral protamine hagedorn.

or not functioning; this is called (probable symptomatic hypoglycemia). Besides, patients with poor glycemic control and persistently high blood sugar levels could experience hypoglycemia at blood glucose level > 70 mg/dL (3.9 mmol/L), this is called (relative hypoglycemia)<sup>[5]</sup>.

By the present study it was found that factors related to meal (missed meal, delayed meal and eating less amount of food in meals) were the most common precipitating factors of hypoglycemic events, this is agreed with what is known by most literatures<sup>[2,8,9,11]</sup>.

Nocturnal hypoglycemia is a dangerous problem in patients with T2DM on insulin, if it is severe enough; it may lead to death or serious neurological impairment, it occurs in about two thirds of the studied patients. Eating less amount of food in dinner and use of bed time intermediate acting NPH human insulin may contribute to nocturnal hypoglycemia<sup>[2,8,12]</sup>.

Hypoglycemia unawareness occurs in a minority of the studied patients who report episodes of severe hypoglycemia that necessitate medical management in hospitals. Long standing T2DM and recurrent hypo-

glycemic episodes are possible risk factors<sup>[11]</sup>. These results agreed with those study of Akram *et al.*<sup>[13]</sup>.

No relation was found in the present study of hypoglycemia to age. The same findings were obtained by Davis *et al.*<sup>[14]</sup>, while contradictory results were reported in other studies that concluded aging as an important risk factor of hypoglycemia<sup>[15-18]</sup>.

This may be due to that elderly people constitute a small proportion of the studied patients (only 6.5%).

Although in our study females predominates males; no association was found between gender and hypoglycemia, several recent studies support our findings<sup>[14,19,20]</sup>.

There is a significant association between hypoglycemia and level of education at the level of univariate analysis ( $P < 0.05$ ) but this association has disappeared at the level of logistic regression. Hypoglycemia is more prevalent among illiterate patients or those with lower than secondary school qualification. Low educational attainment may mean less understanding and carelessness regarding the dangerous complications of hypoglycemia and the importance of adherence to



**Table 5** Relation of hypoglycemia during the last 3 mo with availability, frequency of self-monitoring of blood glucose, common co-morbidities, and concomitant medication use (other than OHD)

	Hypoglycemia in the last 3 mo		Total (n)	P value
	Yes n (%)	No n (%)		
Availability of SMBG				
Available and used	124 (75.2)	41 (24.8)	165	0.996
Not available	102 (75.6)	33 (24.4)	135	
Available and not used	27 (75.0)	9 (25.0)	36	
Frequency of SMBG use				
Once/mo	19 (79.2)	5 (20.8)	24	0.164
1-2 times/wk	48 (67.6)	23 (32.4)	71	
Once daily	23 (74.2)	8 (25.8)	31	
Twice daily	8 (88.9)	1 (11.1)	9	
Thrice daily	2 (50.0)	2 (50.0)	4	
According to patients condition	25 (89.3)	3 (10.7)	28	
Common co-morbidities				
HTN	106 (72.1)	41 (27.9)	147	0.232
IHD	50 (86.2)	8 (13.8)	58	0.034
CVA	15 (75.0)	5 (25.0)	20	0.975
Amputation	7 (70.0)	3 (30.0)	10	0.693
Diabetic foot	72 (79.1)	19 (20.9)	91	0.322
CKD	66 (73.3)	24 (26.7)	90	0.614
PNP	235 (77.6)	68 (22.4)	303	0.004
Insulin use by other family members				
At home	21 (80.8)	5 (19.2)	26	0.764
Outside home	42 (76.4)	13 (23.6)	55	
Non	190 (74.5)	65 (25.5)	255	
Concomitant medication use (other than OHD)				
Yes	215 (74.1)	75 (25.9)	290	0.216

SMBG: Self-monitoring of blood glucose; HTN: Hypertension; IHD: Ischemic heart diseases; CVA: Cerebrovascular accidents; CKD: Chronic kidney disease; PNP: Peripheral neuropathy.

the treatment plan and those patients may be unable to adjust insulin doses according to their daily activities or meals. These results did agree with results found by ACCORD<sup>[21]</sup>.

There was no significant association between marital status and hypoglycemia in our study, a result that agrees with what was found by Bruce *et al*<sup>[22]</sup> but contradicts the results of Akram *et al*<sup>[13]</sup> in that being married is a risk factor for hypoglycemia.

Although there is no significant association between residence and hypoglycemia at univariate analysis; a strong negative association does exist at the level of multivariate analysis ( $P < 0.05$ ) which implies that patient from periphery of Basrah (outside the major city of Basrah) experienced hypoglycemia more than patients from Basrah city, this could be explained partially by difficult access to these patients to FDEMC according to their appointments to adjust their insulin regimens and partly because patients living in rural areas may have relatively low education than those living in Basrah city which is found to be significantly associated with hypoglycemia in our study and others<sup>[21,23]</sup>.

No relationship was found in our study between duration of DM and hypoglycemia, the same is found by some studies<sup>[14,19,22]</sup>. But not in ACCORD<sup>[21]</sup> which is a large randomized controlled trial that follow-up large number of patients for several years most of them were elderly and have longer duration of diabetes and Akram

*et al*<sup>[13]</sup> who found that the risk of hypoglycemia increased progressively when the duration of diabetes was more than 16 years and United Kingdom Hypoglycemia Study Group<sup>[10]</sup> who found that risk of hypoglycemia in insulin treated patients increased after 5 years of therapy. The present study is a cross sectional one that investigated retrospectively the experience of hypoglycemia among diabetic patients in the last 3 and 12 mo and more than half of them were diagnosed with diabetes for less than 10 years. Thus the duration of diabetes in the studied patients is relatively short and could not allow the effect of duration to be identified.

In addition, no significant association between types of insulins studied [regular human, premix human (70:30) and NPH] with hypoglycemia. The risk of hypoglycemia is seems to be similar with these types. Akram *et al*<sup>[13]</sup> and Miller *et al*<sup>[24]</sup> found that the relationship between type of insulin and risk of severe hypoglycemia is inconsistent.

There is a significant association between the frequency of insulin administration per a day with the experience of hypoglycemia in the preceding 3 mo, which is an established fact in insulin therapy<sup>[25]</sup>.

There is a significant association between presence of IHD and hypoglycemia at the level of univariate analysis ( $P < 0.05$ ) but this association has disappeared at the level of logistic regression. IHD as a part of macrovascular complications of DM is found to be a significant predictor of hypoglycemia<sup>[26]</sup>.

**Table 6** Relation of hypoglycemia during the last 3 mo with body mass index, systolic blood pressure; diastolic blood pressure; hemoglobin A1c; serum creatinine; urine for albumin

Variables	Hypoglycemia in the last 3 mo		Total (n)	P value
	Yes n (%)	No n (%)		
BMI (kg/m <sup>2</sup> )				
Thin or normal (< 25.00)	46 (83.6)	9 (16.4)	55	0.123
Overweight (25.0-29.9)	95 (77.2)	28 (22.8)	123	
Obese (30.00-39.99)	93 (68.9)	42 (31.1)	135	
Morbid obesity (≥ 40)	18 (81.8)	4 (18.2)	22	
Total	252 (75.2)	83 (24.8)	335 <sup>1</sup>	
Systolic blood pressure (mmHg)				
Normal (< 130)	91 (79.8)	23 (20.2)	114	0.157
Prehypertension (130-139)	62 (79.5)	16 (20.5)	78	
Stage 1 hypertension (140-159)	76 (71.0)	31 (29.0)	107	
Stage 2 hypertension (≥ 160)	24 (64.9)	13 (35.1)	37	
Total	25 (375.3)	83 (24.7)	336	
Diastolic blood pressure (mmHg)				
Normal (< 80)	63 (78.8)	17 (21.2)	80	0.792
Pre-hypertension (80-89)	148 (74.4)	51 (25.6)	199	
Stage 1 hypertension (90-99)	38 (74.5)	13 (25.5)	51	
Stage 2 hypertension (≥ 100)	4 (66.7)	2 (33.3)	6	
Total	253 (75.3)	83 (24.7)	336	
HbA1c (%)				
< 7.0	10 (83.3)	2 (16.7)	12	0.117
7.0-10.0	136 (79.1)	36 (20.9)	172	
10.1-13.0	85 (73.3)	31 (26.7)	116	
> 13.0	22 (61.1)	14 (38.9)	36	
Total	253 (75.3)	83 (24.7)	336	
Serum creatinine (mg/dL)				
< 0.7	66 (72.5)	25 (27.5)	91	0.632
0.7-1.4	167 (75.9)	53 (24.1)	220	
> 1.4	3 (100.0)	0 (0.0)	3	
Total	236 (75.2)	78 (24.8)	314 <sup>2</sup>	
Urine for albumin (positive)	65 (75.6)	21 (24.4)	86	0.947
Total	235 (75.3)	77 (24.7)	312 <sup>3</sup>	

<sup>1</sup>BMI had not been measured for one patient due to bilateral lower limb amputation; <sup>2,3</sup>Unequal numbers because some of the patients did not complete their investigations. BMI: Body mass index; HbA1c: Hemoglobin A1c.

Furthermore a significant association between the presence of PNP and risk of hypoglycemia ( $P < 0.05$ ) both at the level of univariate and logistic regression analyses was found. PNP may reflect advanced diabetes and its associated microvascular complications, *e.g.*, autonomic neuropathy. This result agrees with what was found by Miller *et al.*<sup>[27]</sup>

No relation was found between family/social support, vision and mobility with risk of hypoglycemia. These factors were not applied as risk factors in the previously mentioned large randomized controlled trials UKPDS<sup>[28]</sup>, ACCORD<sup>[21]</sup>, VADT<sup>[29]</sup>, United Kingdom Hypoglycemia Study Group<sup>[10]</sup>. We explored their effect as indicators of severity of diabetes and thus we assumed a patient who needed support and restricted vision and mobility was likely to develop hypoglycemia.

At the level of univariate analysis there is a significant relationship between knowledge of hypoglycemia symptoms and hypoglycemia ( $P < 0.05$ ), while at the level of logistic regression also there is a strong positive association with knowledge of hypoglycemia symptoms ( $P < 0.05$ ), *i.e.*, the more knowledge of hypoglycemic symptoms the more hypoglycemia was reported. Although most patients who experience hypoglycemia have prior

knowledge of hypoglycemia symptoms; this knowledge did not protect them from hypoglycemia and this may be due to low education, poor understanding of the importance of adjusting insulin dose and time of injection according to daily activities or the amount and time of meals. Also it may indicate that health education is inadequate, medical practitioners should spent more effort to teach their patients about signs, symptoms, and proper treatment of hypoglycemia, as well has how to prevent it<sup>[30]</sup>.

There is no significant relationship between availability and frequency of SMBG with risk of hypoglycemia; while at the level of logistic regression analysis we found that the availability of SMBG *per se* decreases the risk of hypoglycemia ( $P < 0.05$ ) and frequent use of SMBG associated with more hypoglycemia. Frequent use of SMBG does not protect patients from hypoglycemia nor predict it but probably remind the patient with signals of hypoglycemia, or this may be due to bad storage conditions of the device and strips, high temperature and humidity, absence of hand washing prior to testing. Anyhow, our result agrees with a number of other studies<sup>[31-33]</sup>.

No significant association was found between CVA and amputation with risk of hypoglycemia, same findings

**Table 7 Results of logistic regression showing significant predictors of hypoglycemia in the last 3 mo**

	B	Sig.	Exp (B)
<b>Significant predictors</b>			
Residence	-0.247	0.030	0.782
Knowledge of hypoglycemia symptoms	1.133	0.044	3.104
Availability of SMBG	-0.599	0.030	0.550
Frequency of SMBG	-0.228	0.031	0.796
PNP	-1.391	0.002	0.249
Diastolic blood pressure	-0.046	0.013	0.955
Systolic blood pressure	0.020	0.053	1.020
HbA1c	0.153	0.021	1.165
<b>Non-significant predictors</b>			
Age	0.002	0.960	1.002
Gender	-0.425	0.200	0.654
Education	0.067	0.505	1.069
Duration of DM	-0.019	0.42	0.981
Frequency of insulin administration/d	-0.381	0.259	0.683
Dose of regular insulin	-0.021	0.215	0.979
Dose of premix insulin	-0.027	0.305	0.974
Dose of NPH	-0.022	0.429	0.979
A total dose of insulin	0.018	0.148	1.018
Mobility	0.117	0.440	1.124
HTN	0.594	0.117	1.811
IHD	-0.758	0.081	0.469
CKD	0.307	0.614	1.359
BMI	0.033	0.208	1.033

SMBG: Self-monitoring of blood glucose; HbA1c: Hemoglobin A1c; HTN: Hypertension; IHD: Ischemic heart diseases; CKD: Chronic kidney disease; PNP: Peripheral neuropathy; BMI: Body mass index; DM: Diabetes mellitus; NPH: Neutral protamine hagedorn.

obtained by other studies in that there is no significant association between macrovascular complications of diabetes including CVA and amputation with risk of hypoglycemia<sup>[13,19]</sup>.

Also no significant association was found between diabetic foot and risk of hypoglycemia, this agree with what is found by other studies which suppose that no significant association between microvascular complications of diabetes and risk of hypoglycemia<sup>[14]</sup>.

Although it is agreed that in advanced kidney diseases, insulin excretion from kidneys will decrease and thus the risk of hypoglycemia will increase<sup>[14,27]</sup>. No significant association was found between, CKD and risk of hypoglycemia, this looks similar to what is found by other studies<sup>[19,24]</sup>. There was no significant association between BMI and hypoglycemia, similar results were found by other studies<sup>[14,19,24]</sup>.

No significant association was found between systolic and diastolic blood pressure and risk of hypoglycemia at level of univariate analysis but there is a significant positive association between diastolic blood pressure and risk of hypoglycemia at the level of logistic regression ( $P < 0.05$ ), *i.e.*, as the diastolic blood pressure increase; the risk of hypoglycemia will increase too. Similar results were found by other studies<sup>[34]</sup>. This association may be related to antihypertensive drugs those patients use, namely the ACE inhibitors, which are suggested to be a risk factor for hypoglycemia<sup>[35]</sup>.

Although no significant association was found between

between HbA1c and hypoglycemia at the level of univariate analysis; there was a strong negative association, *i.e.*, the lower the HbA1c; the more the risk of hypoglycemia, this is consistent with what is found by several large studies<sup>[21,28]</sup>. In that intensive glycemic control and HbA1c goal  $< 7$  is associated with increased risk of hypoglycemia (both major and minor).

Taking the results as a whole, particularly the logistic regression analysis, the only residence (rural), knowledge of hypoglycemia symptoms, availability and increasing frequency of SMBG, the presence of the PNP, high diastolic blood pressure and low HbA1c were significant and independent predictors of hypoglycemia. All other studied variables were not predictors.

### Limitations of the study

Although every patient entering this center (FDEMC) on the day of the interview was checked to see if he or she met the inclusion criteria; selection bias cannot be excluded. Another limitation is that a small proportion of patients did not complete their investigations regarding fasting glucose (12.5%), random glucose (12.3%), serum creatinine (6.5%), urine for albumin (7.1%) measurement.

In conclusion, hypoglycemia is very common among insulin treated patients with T2DM Basrah. It was possible to identify a number of important predictors of hypoglycemia.

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## COMMENTS

### Background

Hypoglycemia is one the important barrier for initiating and continuing insulin therapy in type 2 diabetes (T2DM) for patients and doctors. Overcoming this barrier will be fundamental to start insulin at earlier stage.

### Research frontiers

Basrah is one the largest city in Iraq. Data on the hypoglycemia frequency is lacking in Iraq and this city. This study will start to give baseline hypoglycemia frequency in insulin treated patients with T2DM.

### Innovations and breakthroughs

This study showed that some form of hypoglycemia accord in more than three quarter of patients with T2DM treated with insulin. The important predictors of hypoglycemia were residence (rural), knowledge of hypoglycemia symptoms, availability and increasing frequency of self-monitoring blood glucose, the presence of the peripheral neuropathy, high diastolic blood pressure and low hemoglobin A1c (HbA1c).

### Applications

This study provided for the first time data on the frequency of hypoglycemia for the first time in Basrah (Southern Iraq), which seems to be very common.

### Terminology

Hypoglycemia is state of low blood glucose that ranges from mild that can be

self-treated to severe which the need help by the others including the hospital. It can be symptomatic or a symptomatic, documented by blood glucose estimation or not and nocturnal or daytime.

### Peer-review

This paper is well written and the information that contains is a useful tool for physiology and the correlation between miRNAs and impaired fracture healing.

## REFERENCES

- 1 **Vignesh JP**, Mohan V. Hypoglycaemia unawareness. *J Assoc Physicians India* 2004; **52**: 727-732 [PMID: 15839452]
- 2 **Briscoe VJ**, Davis SN. Hypoglycemia in type 1 and type 2 diabetes: physiology, pathophysiology and management. *Clinical Diabetes* 2006; **24**: 115-121 [DOI: 10.2337/diaclin.24.3.115]
- 3 **Seaquist ER**, Anderson J, Childs B, Cryer P, Dagogo-Jack S, Fish L, Heller SR, Rodriguez H, Rosenzweig J, Vigersky R. Hypoglycemia and diabetes: a report of a workgroup of the American Diabetes Association and the Endocrine Society. *J Clin Endocrinol Metab* 2013; **98**: 1845-1859 [PMID: 23589524 DOI: 10.1210/jc.2012-4127]
- 4 Defining and reporting hypoglycemia in diabetes: a report from the American Diabetes Association Workgroup on Hypoglycemia. *Diabetes Care* 2005; **28**: 1245-1249 [PMID: 15855602 DOI: 10.2337/diacare.28.5.1245]
- 5 **Fritsche A**, Hahn A, Landgraf W, Häring HU. Incidence of Hypoglycemia in Patients with Type 2 Diabetes - A Subgroup Analysis from the GINGER study. *European Endocrinology* 2013; **9**: 1-3 [DOI: 10.17925/EE.2013.09.01.1]
- 6 **Donnelly LA**, Morris AD, Frier BM, Ellis JD, Donnan PT, Durrant R, Band MM, Reekie G, Leese GP. Frequency and predictors of hypoglycaemia in Type 1 and insulin-treated Type 2 diabetes: a population-based study. *Diabet Med* 2005; **22**: 749-755 [PMID: 15910627 DOI: 10.1111/j.1464-5491.2005.01501.x]
- 7 **Henderson JN**, Allen KV, Deary IJ, Frier BM. Hypoglycaemia in insulin-treated Type 2 diabetes: frequency, symptoms and impaired awareness. *Diabet Med* 2003; **20**: 1016-1021 [PMID: 14632703]
- 8 **Cryer PE**. The barrier of hypoglycemia in diabetes. *Diabetes* 2008; **57**: 3169-3176 [PMID: 19033403 DOI: 10.2337/db08-1084]
- 9 **Fowler MJ**. Hypoglycemia. *Clinical Diabetes* 2008; **26**: 170-173 [DOI: 10.2337/diaclin.26.4.170]
- 10 **UK Hypoglycaemia Study Group**. Risk of hypoglycaemia in types 1 and 2 diabetes: effects of treatment modalities and their duration. *Diabetologia* 2007; **50**: 1140-1147 [PMID: 17415551 DOI: 10.1007/s00125-007-0599-y]
- 11 **Zammit NN**, Frier BM. Hypoglycemia in type 2 diabetes: pathophysiology, frequency, and effects of different treatment modalities. *Diabetes Care* 2005; **28**: 2948-2961 [PMID: 16306561 DOI: 10.2337/diacare.28.12.2948]
- 12 **Lingvay I**. Hypoglycemia in Type 2 Diabetes - Consequences and Risk Assessment. *US Endocrinology* 2011; **7**: 95-102 [DOI: 10.17925/USE.2011.07.02.95]
- 13 **Akram K**, Pedersen-Bjergaard U, Carstensen B, Borch-Johnsen K, Thorsteinsson B. Frequency and risk factors of severe hypoglycaemia in insulin-treated Type 2 diabetes: a cross-sectional survey. *Diabet Med* 2006; **23**: 750-756 [PMID: 16842479 DOI: 10.1111/j.1464-5491.2006.01880.x]
- 14 **Davis TM**, Brown SG, Jacobs IG, Bulsara M, Bruce DG, Davis WA. Determinants of severe hypoglycemia complicating type 2 diabetes: the Fremantle diabetes study. *J Clin Endocrinol Metab* 2010; **95**: 2240-2247 [PMID: 20305006 DOI: 10.1210/jc.2009-2828]
- 15 **Meneilly GS**, Cheung E, Tuokko H. Altered responses to hypoglycemia of healthy elderly people. *J Clin Endocrinol Metab* 1994; **78**: 1341-1348 [PMID: 8200936 DOI: 10.1210/jcem.78.6.8200936]
- 16 **Meneilly GS**, Cheung E, Tuokko H. Counterregulatory hormone responses to hypoglycemia in the elderly patient with diabetes. *Diabetes* 1994; **43**: 403-410 [PMID: 8314012]
- 17 **Matyka K**, Evans M, Lomas J, Cranston I, Macdonald I, Amiel SA. Altered hierarchy of protective responses against severe hypoglycemia in normal aging in healthy men. *Diabetes Care* 1997; **20**: 135-141 [PMID: 9118760 DOI: 10.2337/diacare.20.2.135]
- 18 **Schwartz AV**, Vittinghoff E, Sellmeyer DE, Feingold KR, de Rekeneire N, Strotmeyer ES, Shorr RI, Vinik AI, Odden MC, Park SW, Faulkner KA, Harris TB. Diabetes-related complications, glycemic control, and falls in older adults. *Diabetes Care* 2008; **31**: 391-396 [PMID: 18056893 DOI: 10.2337/dc07-1152]
- 19 **Sarkar U**, Karter AJ, Liu JY, Moffet HH, Adler NE, Schillinger D. Hypoglycemia is more common among type 2 diabetes patients with limited health literacy: the Diabetes Study of Northern California (DISTANCE). *J Gen Intern Med* 2010; **25**: 962-968 [PMID: 20480249 DOI: 10.1007/s11606-010-1389-7]
- 20 **Shen JJ**, Washington EL. Identification of diabetic complications among minority populations. *Ethn Dis* 2008; **18**: 136-140 [PMID: 18507263]
- 21 **Gerstein HC**, Miller ME, Byington RP, Goff DC, Bigger JT, Buse JB, Cushman WC, Genuth S, Ismail-Beigi F, Grimm RH, Probstfield JL, Simons-Morton DG, Friedewald WT. Effects of intensive glucose lowering in type 2 diabetes. *N Engl J Med* 2008; **358**: 2545-2559 [PMID: 18539917 DOI: 10.1056/NEJMoa0802743]
- 22 **Bruce DG**, Davis WA, Casey GP, Clarnette RM, Brown SG, Jacobs IG, Almeida OP, Davis TM. Severe hypoglycaemia and cognitive impairment in older patients with diabetes: the Fremantle Diabetes Study. *Diabetologia* 2009; **52**: 1808-1815 [PMID: 19575177 DOI: 10.1007/s00125-009-1437-1]
- 23 **Zoungas S**, Patel A, Chalmers J, de Galan BE, Li Q, Billot L, Woodward M, Ninomiya T, Neal B, MacMahon S, Grobbee DE, Kengne AP, Marre M, Heller S. Severe hypoglycemia and risks of vascular events and death. *N Engl J Med* 2010; **363**: 1410-1418 [PMID: 20925543 DOI: 10.1056/NEJMoa1003795]
- 24 **Miller CD**, Phillips LS, Ziemer DC, Gallina DL, Cook CB, El-Kebbi IM. Hypoglycemia in patients with type 2 diabetes mellitus. *Arch Intern Med* 2001; **161**: 1653-1659 [PMID: 11434798]
- 25 **Joshi S**, Joshi P. A review of insulin and insulin regimens in type 2 diabetes. *SA Fam Pract* 2009; **51**: 97-102 [DOI: 10.1080/20786204.2009.10873820]
- 26 **Quilliam BJ**, Simeone JC, Ozbay AB. Risk factors for hypoglycemia-related hospitalization in patients with type 2 diabetes: a nested case-control study. *Clin Ther* 2011; **33**: 1781-1791 [PMID: 22018449 DOI: 10.1016/j.clinthera.2011.09.020]
- 27 **Miller ME**, Bonds DE, Gerstein HC, Seaquist ER, Bergenstal RM, Calles-Escandon J, Childress RD, Craven TE, Cuddihy RM, Dailey G, Feinglos MN, Ismail-Beigi F, Largay JF, O'Connor PJ, Paul T, Savage PJ, Schubart UK, Sood A, Genuth S. The effects of baseline characteristics, glycaemia treatment approach, and glycated haemoglobin concentration on the risk of severe hypoglycaemia: post hoc epidemiological analysis of the ACCORD study. *BMJ* 2010; **340**: b5444 [PMID: 20061360 DOI: 10.1136/bmj.b5444]
- 28 Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. *Lancet* 1998; **352**: 837-853 [PMID: 9742976]
- 29 **Duckworth W**, Abraira C, Moritz T, Reda D, Emanuele N, Reaven PD, Zieve FJ, Marks J, Davis SN, Hayward R, Warren SR, Goldman S, McCarren M, Vitek ME, Henderson WG, Huang GD. Glucose control and vascular complications in veterans with type 2 diabetes. *N Engl J Med* 2009; **360**: 129-139 [PMID: 19092145 DOI: 10.1056/NEJMoa0808431]
- 30 **American Diabetes Association**. Standards of medical care in diabetes--2008. *Diabetes Care* 2008; **31** Suppl 1: S12-S54 [PMID: 18165335 DOI: 10.2337/dc08-S012]
- 31 **Ginsberg BH**. Factors affecting blood glucose monitoring: sources of errors in measurement. *J Diabetes Sci Technol* 2009; **3**: 903-913 [PMID: 20144340]
- 32 **Bamberg R**, Schulman K, MacKenzie M, Moore J, Olchesky S. Effect of adverse storage conditions on performance of glucometer test strips. *Clin Lab Sci* 2005; **18**: 203-209 [PMID: 16315736]
- 33 **Cox DJ**, Gonder-Frederick L, Ritterband L, Clarke W, Kovatchev



- BP. Prediction of severe hypoglycemia. *Diabetes Care* 2007; **30**: 1370-1373 [PMID: 17363757 DOI: 10.2337/dc06-1386]
- 34 **Hsu PF**, Sung SH, Cheng HM, Yeh JS, Liu WL, Chan WL, Chen CH, Chou P, Chuang SY. Association of clinical symptomatic hypoglycemia with cardiovascular events and total mortality in type 2 diabetes: a nationwide population-based study. *Diabetes Care* 2013; **36**: 894-900 [PMID: 23223349 DOI: 10.2337/dc12-0916]
- 35 **Vue MH**, Setter MS. Drug-Induced Glucose Alterations Part 1: Drug-Induced Hypoglycemia. *Diabetes Spectrum* 2011; **24**: 171-177 [DOI: 10.2337/diaspect.24.3.171 ]
- P- Reviewer:** Gómez-Sáez J, Haidara M, Savopoulos C, Romani A  
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## Response to comment on: Statin use and risk of diabetes mellitus

Bharti Chogtu, Rahul Magazine, Kurady Laxminarayana Bairy

Bharti Chogtu, Kurady Laxminarayana Bairy, Department of Pharmacology, Kasturba Medical College, Manipal University, Manipal 576104, Karnataka, India

Rahul Magazine, Department of Pulmonary Medicine, Kasturba Medical College, Manipal University, Manipal 576104, Karnataka, India

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**Correspondence to:** Dr. Bharti Chogtu, MD, Associate Professor, Department of Pharmacology, Kasturba Medical College, Manipal University, SH 65, Madhav Nagar, Manipal 576104, Karnataka, India. [bhartimagazine@gmail.com](mailto:bhartimagazine@gmail.com)  
Telephone: +91-0820-2922582

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### Abstract

In letter to the editor "Comment on: Statin use and

risk of diabetes mellitus" authors found the statement "pravastatin 40 mg/d reduced the risk of diabetes by 30% in West of Scotland Coronary Prevention study" erroneous. As per our opinion the statement is right but had been referenced incorrectly.

**Key words:** Pravastatin; Diabetes mellitus

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**Core tip:** The statement "Pravastatin has shown to decrease the risk of developing diabetes by 30%" is correct and had been wrongly referenced in "Statin use and risk of diabetes mellitus" by Chogtu *et al.*

Chogtu B, Magazine R, Bairy KL. Response to comment on: Statin use and risk of diabetes mellitus. *World J Diabetes* 2016; 7(18): 481-482 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v7/i18/481.htm> DOI: <http://dx.doi.org/10.4239/wjd.v7.i18.481>

### TO THE EDITOR

We thank Eren *et al* for showing interest in the review article "statin use and risk of diabetes mellitus"<sup>[1]</sup>. Eren *et al*<sup>[2]</sup> have pointed to an apparent factual error in the following statement: "pravastatin 40 mg/d reduced the risk of diabetes by 30% in West of Scotland Coronary Prevention study (WOSCOPS)". However, in our opinion, this statement is correct if we refer to WOSCOPS 2001, in which authors have put forth that pravastatin in a dose of 40 mg/d resulted in a 30% reduction ( $P = 0.042$ ) in the risk of diabetes<sup>[3]</sup>. Haffner<sup>[4]</sup> in an editorial in the same issue of *Circulation* has also alluded to the fact that Pravastatin reduced incidence of diabetes by 30% - though with a caveat that these results should be cautiously interpreted as the statistical significance in WOSCOPS 2001 was modest.

Regarding the review article “Statin use and risk of diabetes mellitus” the error is not in the statement but in quoting the reference. We should have referenced it as Freeman *et al*<sup>[3]</sup> rather than Kotseva *et al*<sup>[5]</sup>. We again thank Eren *et al*<sup>[2]</sup> to bring this error to our notice.

## REFERENCES

- 1 **Chogtu B**, Magazine R, Bairy KL. Statin use and risk of diabetes mellitus. *World J Diabetes* 2015; **6**: 352-357 [PMID: 25789118 DOI: 10.4239/wjd.v6.i2.352]
- 2 **Eren MA**, Sabuncu T, Karaaslan H. Comment on: Statin use and risk of diabetes mellitus. *World J Diabetes* 2016; **7**: 175-176 [PMID: 27114756 DOI: 10.4239/wjd.v7.i8.175]
- 3 **Freeman DJ**, Norrie J, Sattar N, Neely RD, Cobbe SM, Ford I, Isles C, Lorimer AR, Macfarlane PW, McKillop JH, Packard CJ, Shepherd J, Gaw A. Pravastatin and the development of diabetes mellitus: evidence for a protective treatment effect in the West of Scotland Coronary Prevention Study. *Circulation* 2001; **103**: 357-362 [PMID: 11157685 DOI: 10.1161/01.CIR.103.3.357]
- 4 **Haffner SM**. Do interventions to reduce coronary heart disease reduce the incidence of type 2 diabetes? A possible role for inflammatory factors. *Circulation* 2001; **103**: 346-347 [PMID: 11157682 DOI: 10.1161/01.CIR.103.3.346]
- 5 **Kotseva K**, Wood D, De Backer G, De Bacquer D, Pyörälä K, Keil U. Cardiovascular prevention guidelines in daily practice: a comparison of EUROASPIRE I, II, and III surveys in eight European countries. *Lancet* 2009; **373**: 929-940 [PMID: 19286092 DOI: 10.1016/S0140-6736(09)60330-5]

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# Nutrition, insulin resistance and dysfunctional adipose tissue determine the different components of metabolic syndrome

Juan Antonio Paniagua

Juan Antonio Paniagua, Insulin Resistance, Metabolism and Adipose Tissue Unit, Maimonides Institute of Biomedical Research, University Hospital Reina Sofia, 14004 Cordoba, Spain

Juan Antonio Paniagua, Endocrinology and Nutrition Services, University Hospital Reina Sofia, 14004 Cordoba, Spain

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**Correspondence to:** Juan Antonio Paniagua, PhD, Insulin Resistance, Metabolism and Adipose Tissue Unit, Maimonides Institute of Biomedical Research, University Hospital Reina Sofia, Avda Menendez Pidal, s/n, 14004 Cordoba, Spain. [japaniaguag@yahoo.es](mailto:japaniaguag@yahoo.es)  
 Telephone: +34-95-7011235  
 Fax: +34-95-7011235

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## Abstract

Obesity is an excessive accumulation of body fat that may be harmful to health. Today, obesity is a major public health problem, affecting in greater or lesser proportion all demographic groups. Obesity is estimated by body mass index (BMI) in a clinical setting, but BMI reports neither body composition nor the location of excess body fat. Deaths from cardiovascular diseases, cancer and diabetes accounted for approximately 65% of all deaths, and adiposity and mainly abdominal adiposity are associated with all these disorders. Adipose tissue could expand to inflexibility levels. Then, adiposity is associated with a state of low-grade chronic inflammation, with increased tumor necrosis factor- $\alpha$  and interleukin-6 release, which interfere with adipose cell differentiation, and the action pattern of adiponectin and leptin until the adipose tissue begins to be dysfunctional. In this state the subject presents insulin resistance and hyperinsulinemia, probably the first step of a dysfunctional metabolic system. Subsequent to central obesity, insulin resistance, hyperglycemia, hypertriglyceridemia, hypoalbuminemia, hypertension and fatty liver are grouped in the so-called metabolic syndrome (MetS). In subjects with MetS an energy balance is critical to maintain a healthy body weight, mainly limiting the intake of high energy density foods (fat). However, high-carbohydrate rich (CHO) diets increase postprandial peaks of insulin and glucose. Triglyceride-rich lipoproteins are also increased, which interferes with reverse cholesterol transport lowering high-density lipoprotein cholesterol. In addition, CHO-rich diets could move fat from peripheral to central deposits and reduce adiponectin activity in peripheral adipose tissue. All these are improved with monounsaturated fatty acid-rich diets. Lastly, increased portions of  $\omega$ -3 and  $\omega$ -6 fatty acids also decrease triglyceride levels, and complement the healthy diet that is recommended in patients with MetS.

**Key words:** Obesity; Metabolic syndrome; Metabolism; Adipokines; Insulin resistance; Lipotoxicity and nutrition

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**Core tip:** Central obesity, the insulin resistance, hyperglycemia, hypertriglyceridemia, hypoalphalipoproteinemia, hypertension and fatty liver are grouped in the so-called metabolic syndrome (MetS). In subjects with MetS an energy balance is critical to maintain a healthy body weight, mainly limiting the intake of high energy density foods. However, high-carbohydrate rich (CHO) diets increase postprandial peaks of insulin and glucose. Triglyceride-rich lipoproteins are also increased, which interferes with reverse cholesterol transport lowering high-density lipoprotein cholesterol. In addition, CHO-rich diets could move fat from peripheral to central deposits and reduce adiponectin activity in peripheral adipose tissue. All these are improved with monounsaturated fatty acid-rich diets. Lastly, increased portions of  $\omega$ -3 and  $\omega$ -6 fatty acids also decrease triglyceride levels, and complement the healthy diet that is recommended in patients with MetS.

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## INTRODUCTION

Overweight and obesity are an excessive accumulation of body fat that may be harmful to health. Today, obesity is a major public health problem, affecting in greater or lesser proportion all demographic groups. Obesity is estimated by body mass index (BMI) in a clinical setting, but BMI reports neither body composition nor the location of excess body fat. People with normal weight but high body fat percentages could have a cardiovascular risk equal to that of people with obesity.

Deaths from cardiovascular diseases (CVD), cancer and diabetes accounted for approximately 65% of all deaths, and general adiposity and mainly abdominal adiposity are associated with increased risk of death for all these disorders. Adipose tissue could expand to levels of inflexibility. Then, adiposity is associated with a state of low-grade chronic inflammation, with increased tumor necrosis factor (TNF)- $\alpha$  and interleukin (IL)-6 release, which interfere with adipose cell differentiation, and the action pattern of adiponectin and leptin until the adipose tissue begins to be dysfunctional. In this state the subject presents insulin resistance (IR) and hyperinsulinemia, probably the first step of a dysfunctional metabolic system. Subsequent to central obesity, insulin resistance, hyperglycemia, hypertriglyceridemia, hypoalphalipo-

proteinemia, hypertension and fatty liver are grouped in the so-called metabolic syndrome (MetS).

In subjects with MetS an energy balance is critical to maintain a healthy body weight, mainly limiting high energy density foods. The first factor to be avoided in the prevention of MetS is obesity, and the percentage of fat in the diet has traditionally been associated with the development of obesity. However, it is well established that the type of fat consumed could be more decisive than the total amount of fat consumed when we only look at changes in body composition and distribution of adipose tissue. In addition, insulin resistance is a feature of MetS and is associated with other components of the syndrome. The beneficial impact of fat quality on insulin sensitivity (IS) was not seen in individuals with a high fat intake (> 37E%). Other dietary factors that can influence various components of MetS, like postprandial glycemic and insulin levels, triglycerides and high-density lipoprotein (HDL)-C levels, weight regulation and body composition, as well as fatty liver, are the glycemic load (GL) and the excess of fructose, and amount of dietary fiber content of food eaten. The increased levels of triglycerides associated with hypoalphalipoproteinemia are a feature of insulin resistance and MetS, and increase cardiovascular risk regardless of low-density lipoprotein (LDL) cholesterol levels.

High-carbohydrate rich (CHO) diets increase postprandial peaks of insulin and glucose. Triglyceride-rich lipoproteins are also increased, which interferes with reverse cholesterol transport lowering HDL cholesterol. In addition, CHO-rich diets could move fat from peripheral to central deposits and reduce adiponectin activity in peripheral adipose tissue. All these are improved with monounsaturated fatty acids (MUFA)-rich diets.

The American Diabetes Association (ADA) recommends an intake of dietary fiber of 20 to 35 g/d mainly because of the cholesterol-lowering and glucose-lowering effects of soluble fiber. However, more beneficial effects of a higher intake of dietary fiber, particularly of the soluble type, above the level recommended by the ADA, were reported to improve glycemic control, decreases hyperinsulinemia, and lower plasma lipid concentrations in patients with type 2 diabetes.

Lastly, the prevalence of enlarged waist circumference, hypertension and hypertriacylglycerolemia were reduced after the isoenergetic low fat high complex carbohydrates (LFHCC) supplemented with  $\omega$ -3 diet. Thus, the prevalence of MetS fell by 20.5% after LFHCC  $\omega$ -3 diet compared with the high saturated fatty acids (HSFA) (10.6%), high MUFA (HMUFA) (12%) or LFHCC (10.4%) diets. Therefore, increased fish intake instead of meat portions increases  $\omega$ -3 fatty acids, and moderate portions of dried fruits (walnuts) increases  $\omega$ -6, could complement the healthy diet that is recommended in patients with MetS.

In summary, an equilibrate calory diet, low in animal fat, sugar and fructose, high in MUFA and polyunsaturated fatty acids (PUFA), fresh vegetables high

in fiber, and with moderate complex carbohydrates portions, could improve weight loss, lower postprandial glucose and insulin levels, and triglyceride levels could also decrease, and, eventually, increased HDL cholesterol levels are observed.

The maintenance of an ideal body weight, usually established between 18 and 25 years of age, requires achieving a life-long energy balance, where the amount of energy intake must equal the amount of energy expended. However, in the study of obesity in humans, if we look at only the imbalance between energy intake and energy expenditure, we have failed in its clinical application<sup>[1,2]</sup>. In humans, obesity depends on multiple factors apart from diet, like age and stage of development, genes and epigenetic factors, physical activity, environment, level of instruction and nutrition education, as well as several diseases that alter both physical and psychosocial interaction<sup>[3,4]</sup>. Therefore, the increase in overweight and obesity rates are classified as major public health issues, affecting in greater or lesser proportion all demographic groups, irrespective of age, sex, race, education or economic level<sup>[5]</sup>. World Health Organization (WHO) expects the 400 million obese adults worldwide registered in 2005<sup>[6]</sup> to double, and in the United States, obesity has been increasing in both adults and children in the last few years<sup>[7-9]</sup>. The age-standardized rate of death from any cause was generally lowest among subjects with an optimal BMI of 22.5 to 24.9 kg/m<sup>2</sup><sup>[10-12]</sup>.

Recently, it has been observed that death attributed to factors related to high BMI is in fourth place behind deaths from high blood pressure, smoking, and unhealthy diets; and is ahead of deaths attributable to diabetes, physical inactivity, high salt intake, alcoholism and high blood cholesterol levels<sup>[13]</sup>. In addition, epidemiology studies have established associations between food and nutrient intake with specific diseases such as cancer, diabetes and CVD<sup>[14,15]</sup> as well as with obesity, body fat distribution, hypertension, insulin resistance and hyperglycemia<sup>[16-18]</sup>.

Deaths from CVD, cancer and diabetes accounted for up to approximately 65% of all deaths, and general adiposity and central adiposity are related with increased risk of death for all these disorders, shortened life expectancy and causes disability in addition to high economic costs. Where levels of BMI are higher than 25 kg/m<sup>2</sup> a direct relationship with high mortality due to CVD is well established<sup>[3,19-23]</sup>. Cardiovascular disease accounts for approximately 38.5% of all deaths in EE.UU., although have declined substantially since the 1940s and 1960s<sup>[10]</sup>. This trend may be related with several primary prevention activities (for example, smoking cessation, sugar, trans fat and excess of saturated fat ingestion), improved treatment for ischemic acute phase and finally, improved secondary intervention (treatment of hypertension, hyperglycemia and hypercholesterolemia)<sup>[24,25]</sup>. The pattern of obesity may also influence this CVD risk and those with a waist-hip ratio higher to or equal than the average have in general an odds ratio of 3.0 (95%CI: 2.1-4.2) for ischemic cerebrovascular, even when BMI and other risk factors

were adjusted<sup>[26]</sup>. Last, a weight loss of 10% maintained over time in obese subjects may decrease the expected events of coronary and stroke diseases<sup>[27]</sup>.

On the other hand, concurrent with obesity rates during the 90s, there was an increase of diabetes to 61% in the United States (mainly approximately 90%-95% of type 2 diabetes, T2D)<sup>[28]</sup>. The mortality rate directly attributable to diabetes is about 3%, and diabetic patients have 2-4 times higher cardiovascular risk and many die of CVD<sup>[29]</sup>. Obesity and high body fat are related with diabetes in all ethnic groups. In the United States approximately the 70% of T2D prevalence could be attributed to overweight and obesity and, after 10 years, each kilogram gain from ideal body weight, raises the risk by 4.5%<sup>[10]</sup>. However, again "central obesity" is more strongly associated with metabolic complications linked to insulin resistance including diabetes<sup>[30,31]</sup>. For the prevention and treatment of T2D maintenance of a healthy body weight (BMI < 27-30 kg/m<sup>2</sup>) plus physical activity, limit the intake of sugar and saturated fat, and increase the consumption of mono and PUFA, as well as whole grains and fiber<sup>[32-34]</sup>, is recommended.

Finally, all cancers combined accounted for approximately 23% of the total number of deaths<sup>[10]</sup>. The relationship between BMI and a high mortality due to cancer in most specific sites<sup>[12,35]</sup> is well established. Obesity may account for up to 14% of cancer in men and up to 20% of cancer in women, and the risk of death from cancer in people with BMI  $\geq$  40 kg/m<sup>2</sup> increases up to 52% in men and 62% in women as compared with people with normal weight<sup>[36]</sup>. The underlying pathophysiological mechanisms that may be attributed to increase cancer rates are uncertain but can involve higher circulating levels of glucose, low-grade inflammatory state in many tissues, increased oxidative stress, as well as the bioavailability of hormones, mainly insulin, estrogens and androgens.

After obesity is developed most subjects present IR and hyperinsulinemia, probably the first step of a dysfunctional metabolic system. Subjects with more central obesity present a higher risk of IR, hyperglycemia, hypertriglyceridemia, hypoalbuminoproteinemia, hypertension and fatty liver, and different combination are grouped in so-called MetS. In subjects with MetS achieving an energy balance is critical to maintain a healthy body weight, limiting the consumption of food with high energy density (fat). However, high-carbohydrate rich (CHO) diets increase postprandial peaks of insulin and glucose, and triglyceride-rich lipoproteins are also increased, which interferes with reverse cholesterol transport lowering HDL cholesterol, and could deposit fat mainly in central deposits and reduce adiponectin activity in peripheral adipose tissue. However, all these were improved with MUFA-rich diets. In addition, food with high fiber content (vegetables and whole-grain) and food rich in  $\omega$ -3 and  $\omega$ -6 fatty acids could improve some components of this dysfunctional metabolic system.

The traditional Mediterranean diet is featured by a moderate to high ingestion of olive oil, a lower density



of calories in the diet, legumes and vegetables, fruit, nuts, and whole cereals; a moderate to higher intake of poultry and fish; a moderate intake of dairy products, but more restrictive in higher caloric density foods such as red and processed meats, and sweets; finally, mainly red wine is drunk with meals<sup>[37]</sup>. Selected subjects at high cardiovascular risk, a Mediterranean style diet supplemented either with extra-virgin olive oil or nuts decrease the incidence of major cardiovascular events<sup>[38]</sup>. Finally, studies of healthy habits in the 50s<sup>[39]</sup> show that physical activity at work, walking and cycling as a means of transport all contributed to overall energy expenditure. However, these physical activities have decreased dramatically in societies today because of sedentary habits at work and in holiday life<sup>[40]</sup>. Thus, dietary habits, a major factor in controlling obesity, are made up of environmental, cultural, economic and technological aspects. These can be modified by agricultural policies that govern prices, extending the range and availability of food and regulating beneficial or harmful dietary components<sup>[41,42]</sup>.

## OBESITY ASSESSMENT

Obesity could be estimated only by measures of the body weight; however, relating body weight to height give us a more accurate measure of obesity<sup>[43]</sup>. The BMI or the Quetelet's index is the measure that is currently used in clinical setting to graduate from the normal weight to obesity in adults, and is estimated by the weight/height ratio squared, and expressed as kg/m<sup>2</sup>. The approach taken by WHO is: (1) BMI between 18-25 kg/m<sup>2</sup> is considered normal weight; (2) BMI between 25-29.9 kg/m<sup>2</sup> is considered overweight; and (3) a BMI greater than or equal to 30 kg/m<sup>2</sup> is defined as obesity<sup>[44,45]</sup>. However, BMI does not give us information about body composition and body fat distribution, neither about individual variations in terms of amounts of lean body mass (fat-free muscle mass), or the pattern of depot on body fat distribution. Thus, the percentage of body fat (BF%) is a better measure as it relates the ratio of total weight of fatty body weight. However, it is more difficult to measure BF% than single BMI, but several methods of varying accuracy and complexity exist<sup>[46]</sup>. In a clinical setting the most commonly used anthropometric indicator of body composition analysis involving two components (body fat and free-fat mass) are estimated from measurements of skinfold thicknesses, that should be measured in several regions, in order to obtain a clearer picture of fat composition<sup>[47]</sup>. In research, the percentage of body fat determined by hydrostatic weighing (body weight by immersion), is the gold standard<sup>[48]</sup>. In addition, the bioelectrical impedance analysis technique is also used to measure body composition, and using a four-terminal bioimpedance analyzer has a prediction error less or equal to the standard anthropometry for estimating body fat<sup>[49]</sup>. Therefore, it is possible to estimate the amount of body water and the proportion of fat-free mass and

by subtracting body fat from total body weight<sup>[50]</sup>. Furthermore, a relatively simple technique to evaluate the total and regional adiposity in an individual involves a study of the whole body with a scan densitometer (dual energy X-ray absorptiometry, DEXA)<sup>[51,52]</sup>.

People with normal weight but high body fat percentages could have a cardiovascular risk equal to that of people with obesity. The range of normal body fat is 2%-5% in men and 10%-13% in women, while the obesity range of body fat percentage is above of 25% in men and 32% in women<sup>[53,54]</sup>. Experimentally, it was observed that BMI = 30 kg/m<sup>2</sup> implies approximately 30% of BF% at 20 years of age but increase to 40% at 60 year in men, while in older women these values were to 40% and 50%, respectively. Therefore, body fat composition changes with age and sex. Body fat percentage for adults can be estimated from the BMI as follows:  $BF\% = 1.2 \times BMI + 0.23 \times \text{age} - 5.4 - (10.8 \times \text{gender})$  (being 0 if gender is male and 1 if female; it differ for children). The correlation between BMI-BF% is  $r = 0.75$  in male and  $r = 0.82$  in females, for all ages<sup>[55]</sup>.

On the other hand, BMI does not report on the location or distribution of excess body fat, it is to say about the distribution of body fat. Central obesity is characterized mainly by excess fat depot in the abdominal area and within the peritoneal cavity and lower expansion of peripheral adipose tissue. In a clinical setting, several parameters can be used to estimate central obesity; the most widely used being the perimeter of waist circumference (WC), hip ratio (HR) and waist-HR (WHR). Recently, the waist-to-height ratio, which relates waist circumference to height, has also been used to identify higher cardiometabolic risk in adults<sup>[56-58]</sup> and children<sup>[59,60]</sup>. This has advantages compared to the BMI, and even with WC and WHR, and a healthy individual should maintain a waist circumference to less than half their height<sup>[61]</sup>. All these parameters help to predict the risk of metabolic diseases such as T2D<sup>[62]</sup>, and could be more effective in the case of CVD<sup>[63]</sup>. In addition, mortality due to any cause was increased with a BMI < 30 when the subjects have a large WC<sup>[64]</sup>. Thus, WC and WHR help to identify high-risk individuals regardless of their BMI<sup>[65]</sup>. The WC range that estimates mainly central adiposity varies with race and it is currently suggested that for individuals of the United States > 88 cm in women and > 102 cm in men; for the European Union  $\geq 80$  cm in women and  $\geq 94$  cm in men; for Chinese and South Asia > 90 cm and for Japanese > 85 cm for both women and men<sup>[66]</sup>. These assessments are used mainly in the clinic, but there are others more complex and more expensive techniques used in research, which are more accurate, such as DEXA, computed tomography (CT), and magnetic resonance imaging (MRI). Distribution of body fat is evaluated by DEXA by automatic scanning of default regions (arms, legs and trunk). The trunk is the area bounded by the horizontal line under the chin, side edges of the ribs and oblique lines through the femoral neck; and leg area includes the area under these oblique lines. This measure has a coefficient of variation of

approximately 2%<sup>[51,52,67]</sup>. Central obesity is composed of abdominal subcutaneous fat and intraabdominal fat, as is seen by MRI and CT. In addition, intraabdominal adipose tissue is composed of visceral adipose tissue (VAT) as omental and mesenteric fat (intraabdominal fat) and retroperitoneal fat mass<sup>[68]</sup>. Finally, single-voxel magnetic resonance spectroscopy is the gold-standard for ectopic fat quantification. Although very similar to MRI, it does not give anatomical information in image form, but gives information about the chemical composition as it is based on chemical shift. The water protons from (-OH) hydroxyl groups have a spectral peak at 4.7 ppm (parts-per-million). However, the triglycerides have the predominant protons from the (-CH<sub>2</sub>)<sub>n</sub> methylene groups<sup>[69,70]</sup>. Finally, ectopic fat is estimated with accurate methods that separate water and fat signals within each voxel (software such as jMRUI). Occasionally other techniques have been used in determining the ectopic fatty tissue including ultrasonography (US), with a highly significant correlation between CT and by US<sup>[71]</sup>.

## ADIPOCYTE AND ADIPOSITY DEVELOPMENT

### *Adipocyte differentiation*

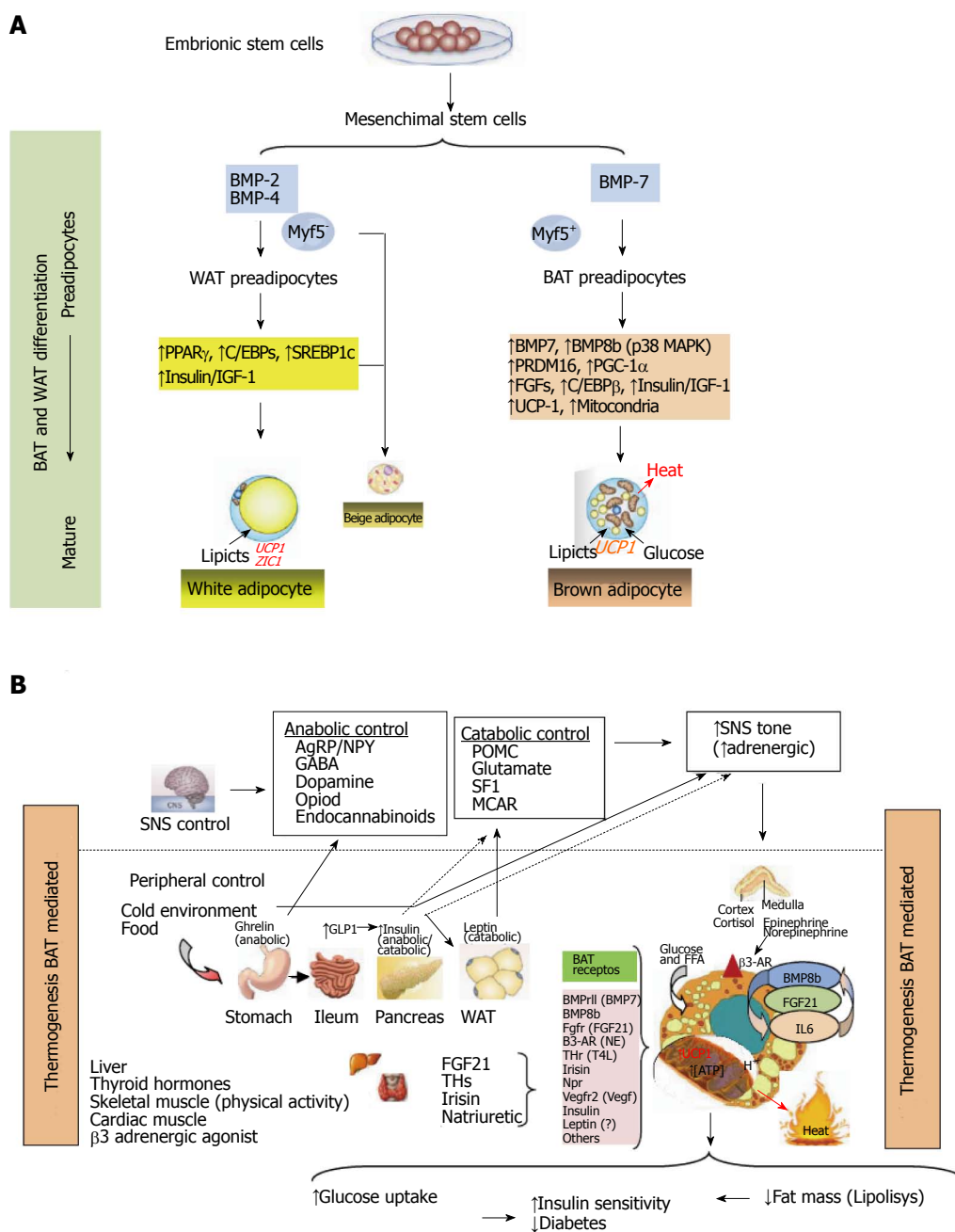
In humans there are two types of well-differentiated adipose tissue, which have different distribution and functions, and are referred to as white adipose tissue (WAT) and brown adipose tissue (BAT) (Figure 1A). The WAT is mainly related to the function of deposit of surplus energy as triacylglycerol (fat), which could be mobilized and offered through hormonal signaling and has a tremendous ability to expand; excess fat storage is associated with mechanical overload and slow to moderate increased risk of metabolic disorders. Mature WAT are characterized by the increased expression of transporters of glucose sensitive to insulin (GLUT4), and enzymes like fatty acid synthase (FAS) and glycerol-2-phosphate dehydrogenase<sup>[72,73]</sup>. By contrast, BAT is involved in thermogenesis functions and thus in energy expenditure and body weight regulation<sup>[74,75]</sup>. In mammals, BAT is the primary site of thermogenesis without accompanying muscle contraction. This function is stimulated by exposure to cold or after lipid-rich calorie food, and this process is called adaptive thermogenesis<sup>[76]</sup>. This thermogenic function of BAT is mediated by the activation of a specific mitochondrial uncoupling protein 1 (UCP1), which is ubiquitous in the inner mitochondrial membrane, uncoupling electron transport of mitochondrial respiration, where the saturation of the production of ATP is dissipated as heat (Figure 1B). The presence of functionally active BAT in rodents has been known for many years. In humans, the first evidence of BAT function was related to the control of body temperature after birth and in early childhood<sup>[77]</sup>. However, several data from adipose tissue samples together with evidence provided by positron emission tomography coupled with computed tomography have established the existence of functionally active

brown adipose tissue in adult humans<sup>[78-81]</sup>. Furthermore, some of these studies have also related data between the size of activation of these sites with BAT and lower BMI, increased basal energy expenditure and decreased onset of diabetes<sup>[82]</sup>. Different amounts of BAT in adult humans can be found in the cervical and supraclavicular<sup>[83]</sup>, and are known as canonical BAT. Although brown adipocytes are also observed infiltrating skeletal muscle and in different areas of WAT<sup>[84]</sup>. Therefore, a third fat cell or new functional adipose tissue is being defined<sup>[85,86]</sup>.

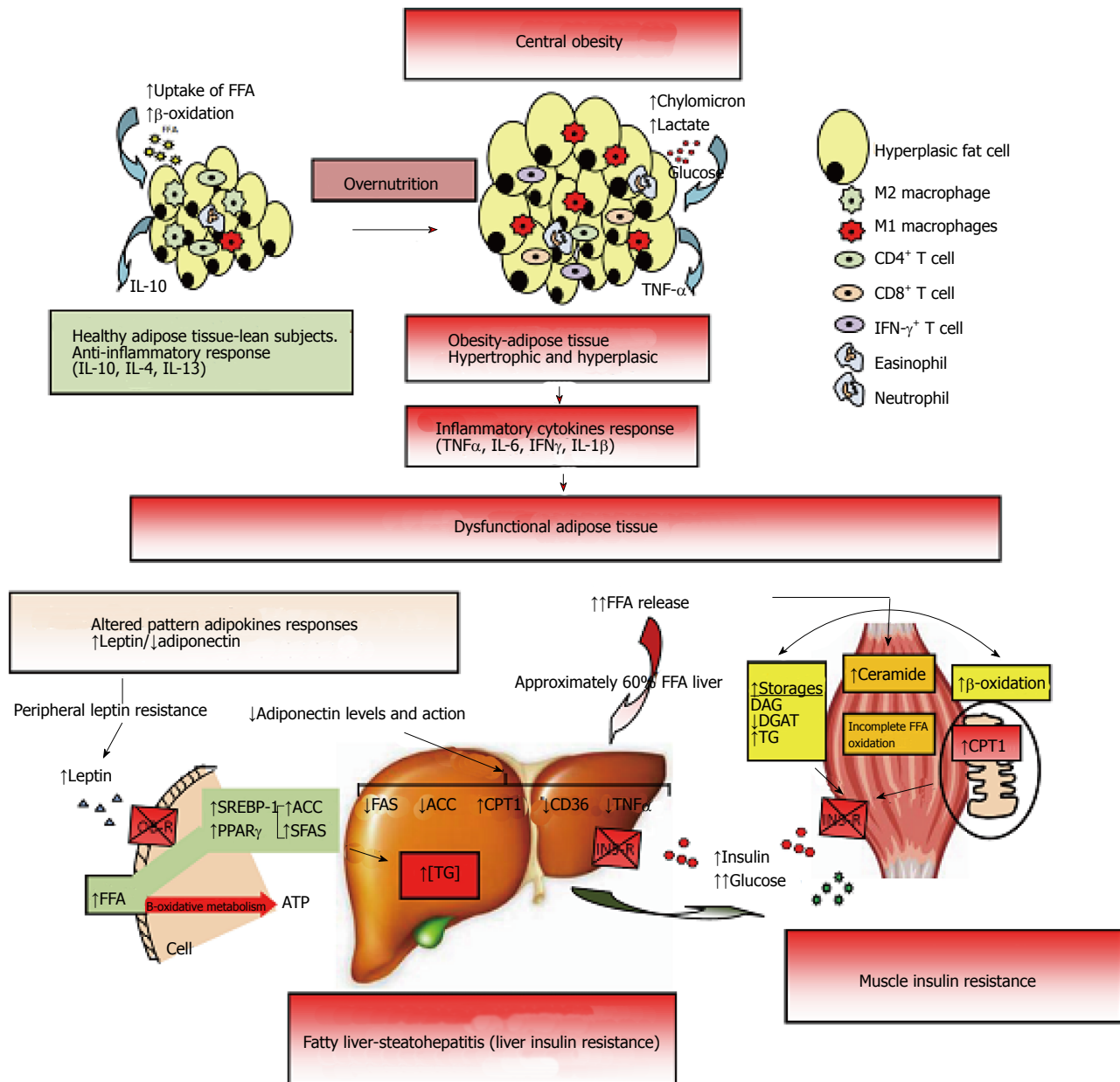
### *Transcriptional signaling of adipocyte formation*

Expansion of WAT in ideal weight or in obesity is not only the result of hypertrophy and/or hyperplasia of adipocytes, but supporting elements like vascular and mesenchymal stromal including immune cells, endothelial cells, and undifferentiated or adipocyte precursor cells (APs) must also be developed. Alterations in vascular tissue development and hypoxia is associated with adipocyte apoptosis and macrophage infiltration, and an appropriate induction of vascular endothelial growth factor A in adipose tissue is essential during expandability of adipose tissue (Figure 2)<sup>[87]</sup>.

The hypertrophy of WAT only depends on its own renewal from APs which remain present during the entire life span and after suitable signaling can form different mature fat cells (Figure 1A)<sup>[88]</sup>. In WAT development several key transcription factors have been identified and among them the binding proteins CCAAT/enhancer (C/EBP) and peroxisome proliferator-activated receptor (PPAR) should be mentioned. Sterol regulatory element binding transcription factor 1 (SREBP1c) has been found as a pro-adipogenic basic helix-loop-helix transcription factor which activates peroxisome proliferator-activated receptor- $\gamma$  (PPAR- $\gamma$ ) expression<sup>[89]</sup> and mediates the induction of lipid biosynthesis by insulin<sup>[90]</sup>. On the other hand, BAT derived from Myf5 + progenitors paraxial mesoderm layer shares a common origin with the development of skeletal myoblasts<sup>[91]</sup>. The development of BAT requires that PRDM16 interacts with either PPAR- $\gamma$  coactivator (PGC-1 $\alpha/\beta$ ) or CtBPs to activate brown genes or the inhibition of several transcription factors that induce WAT, respectively<sup>[92,93]</sup>. In addition, it has been shown that bone morphogenetic protein 7 turn on a complete program of brown adipogenesis involving induction of early key regulatory transcription to brown cells as PRDM16 and PGC-1 $\alpha$ , and increased expression of UCP-1 which is characteristic of brown cells<sup>[94]</sup>. Finally, Myf5 was found to drive the expression of classical BAT depots in retroperitoneal and anterior subcutaneous WATs, and the existence of Myf5 positive cells mixed in WAT has been confirmed<sup>[95]</sup>. The term "beige" has been used to describe those cells that are morphologically identical to white adipocytes, but may be inducible to cells expressing brown adipocytes definitive characteristics of UCP1 activity with  $\beta$ -adrenergic stimulation<sup>[96,97]</sup>. Adipose tissue located in the inguinal area is seen today as the largest and physiologically



**Figure 1 Thermogenesis brown adipose tissue (Bat) mediated.** A: Adipocytes were developed because non adipocytes cells are unable to store calories as fat to meet fuel needs during long periods without eating. If the energy intake is more than energy expenditure, WAT is expanded and leads to obesity. However, a second type of adipose tissue, called BAT was developed especially for energy expenditure (thermogenesis). Today, research in identifying the main genes that control differentiation, development and activation of BAT is highly active, because, activation of BAT, in detriment of WAT, could have anti-obesity effects, which can be utilized to keep the system of fat deposit balanced. In this research, PRDM16, PPAR- $\gamma$  and PGC-1 $\alpha$ , have been identified as the key nodes in the regulation of inducible BAT; B: The thermogenic potential of BAT is controlled by the SNS, which densely innervates brown fat depots. In addition, BAT is activated in response to cold temperatures, hormones and possibly diet. BAT content and activation is highest in children and decreases with age. BAT activation is decreased in fatness, and BAT activity has been inversely correlated to BMI, body fat, and visceral obesity. In humans, BAT amount and activation is higher in women than in men. Of clinical relevance, BAT activation is very low in diabetic patients in comparison with non-diabetic subjects. Thyroid hormones play a main role in control of BAT activation, therefore the cold-induced enhancement of the enzyme 5'-deiodinase type II activity, which deiodinates thyroxine (T<sub>4</sub>) to T<sub>3</sub>. Catecholamines such as norepinephrine binds to  $\beta$ -ARs and induce PGC1 $\alpha$  through p38 MAPK and finally triggers expression of UCP1. Whereas  $\beta$ 1-AR is considered important for proliferation of classical brown adipocyte precursors in response to norepinephrine,  $\beta$ 3-AR plays a major role in thermogenic function of mature brown adipocytes. Another signal, Irisin hormone which comes from muscle to fat tissue, is able to induce a robust browning programme, and mediates the beneficial effects of exercise and could reduce diet-induced obesity and insulin resistance. A more generalized program in the control of adipose tissue is conducted by FGF21 through regulating lipolysis in WAT as well as increasing substrate utilization by increasing fatty acid oxidation in the liver. Last, beige fat cell functions include either a like to "WAT" when energy balance is exceeded, or a like to "BAT" in response to many stimuli similar to BAT activation. WAT: White adipose tissue; BAT: Brown adipose tissue; PRDM16: PR domain containing 16; PPAR- $\gamma$ : Peroxisome proliferator-activated receptor- $\gamma$ ; PGC-1 $\alpha$ : Peroxisome proliferator-activated receptor  $\gamma$  coactivator 1 $\alpha$ ; SNS: Sympathetic nervous system; BMI: Body mass index; FGF21: Fibroblast growth factor 21.



**Figure 2 Dysfunctional adipose tissue.** Early central obesity is associated with a low-grade chronic inflammatory state characterized by slow infiltration of macrophages which are an important source of inflammation of this adipose tissue<sup>[275,276]</sup>. Several macrophage subtypes can be found, and simply put, are divided in pro-inflammatory M1 or alternatively activated M2, although *in vivo* studies reveal a spectrum of macrophage phenotypes<sup>[277]</sup>. Adipocytes and immune cells such as T cells and macrophages participate in the activation and production of inflammatory cytokines<sup>[170,275,278,279]</sup>. The M1 macrophages mainly found in obesity, are induced from precursor M0 macrophages by stimulation of components of bacteria (lipopolysaccharide) and type 1 T-helper (Th1) inflammatory cytokines like IFN- $\gamma$  and TNF- $\alpha$ . The M2 macrophages are activated by type 2 (Th2) cytokines such as IL-4 and IL-13. The M2 macrophages are abundant in adipose tissue of lean subjects and appear to be involved in remodeling, tissue repair, and maintenance of insulin sensitivity through the production and expression of IL-10, IL-1 receptor antagonist, and arginase-1. Whereas M1 macrophages use glucose for energy, M2 macrophages activate the  $\beta$ -oxidation of fatty acids<sup>[277,280]</sup>. Finally, M1 macrophages are the major source of inflammatory cytokines including TNF- $\alpha$  which inhibits adipose cell differentiation by activating Wnt signaling and suppressing expression of PPAR- $\gamma$  transcription factor essential for the development and function of adipocyte, and reducing the effect on stored triglycerides<sup>[281,282]</sup>. The subcutaneous adipose tissue will continue to expand to an equilibrium point. When this capacity is exceeded, glucose and lipid uptake begins to decline and insulin levels are raised to maintain serum glucose in the normal range<sup>[215]</sup>. In addition, when WAT is unable to expand (inflexibility), associated with insulin resistance state, a continuous release of FFA to interstice begins, generating a systemic lipotoxic effect in muscle, liver, etc., (lipotoxicity). The adipose tissue itself begins a slow process of low-level chronic inflammation (macrophages, lymphocytes, etc.) which increases local release of TNF- $\alpha$  and IL-6<sup>[166]</sup>. TNF- $\alpha$  and IL-6 levels are inversely related with peripheral and hepatic glucose-uptake which is insulin-mediated<sup>[283]</sup>. The liver keeps excess uptake of FFA in serum to capacity by joining with glycerol (TAG) and slowly fatty liver is developed (NAFLD). It has been shown that peripheral fatty acids contribute approximately 60% of total TAG stored in the liver, whereas the novo lipogenesis in the liver is approximately 26% and approximately 15% is from the diet<sup>[284]</sup>. On the other hand, leptin levels respond directly to adipose expansion, while adiponectin levels tend to decrease when metabolic syndrome is developed. The elevated leptin levels should increase lipolysis in non-adipose tissues, decreasing excess fatty acids in these cells. However, this action of leptin may be partially blocked by the anabolic effect established by hyperinsulinemia, settling down leptin system dysfunction (peripheral leptin resistance)<sup>[115]</sup>. In addition, the decreased adiponectin levels are inversely related to peripheral glucose uptake and directly related with progressive development of chronic liver disease by fat infiltration. Adiponectin exerts a protective action on liver fat accumulation, favoring lipolysis by promoting the action of CPT-1, while interfering with the action of FAS, ACO and TNF- $\alpha$ , and decreasing the expression and action of CD-36 protein that promotes the transport of fatty acids<sup>[129]</sup>. Finally, both leptin and adiponectin seem to regulate the deposition of fat in insulin-sensitive tissues by increasing fat oxidation. IFN- $\gamma$ : Interferon- $\gamma$ ; TNF- $\alpha$ : Tumor necrosis factor- $\alpha$ ; IL: Interleukin; PPAR- $\gamma$ : Peroxisome proliferator-activated receptor- $\gamma$ ; WAT: White adipose tissue; FFA: Free fatty acids; NAFLD: Non alcoholic fatty liver disease; CPT-1: Carnitine palmitoyltransferase-1; FAS: Fatty acid synthase; ACO: Acyl CoA carboxylase.



most relevant fat depot capable of inducing brought beige adipocytes<sup>[96]</sup>. In addition, it has been observed that in this fat depot the beige mature adipocytes can be interconvert in adipocytes with characteristics typical of white and brown adipocytes, without the need for “*de novo*” cell differentiation from precursors<sup>[97]</sup>. Thus, physiologically this could mean that the rate of lipid storage or lipid oxidation could be adapted and adjusted in response to external stimuli such as a decrease or increase in temperature, but it still requires further investigation.

## EFFECT OF HORMONES AND ADIPOKINES ON ADIPOGENESIS

The adipose tissue can be expanded and developed by many factors such as hormones, growth factors, factors produced by adipose tissue itself (adipokines) and specific effects induced by nutritional factors and some pharmacological components (Figure 1B).

### Hormones and growth factors

**Insulin:** In “*in vitro*” studies, a mixture of dexamethasone, isobutylmethylxanthine and insulin is regularly used to generate well-differentiated adipose tissue, insulin being the most potent of the three factors. Insulin within the physiological range induces lipogenesis and insulin receptor is required for adipocyte differentiation<sup>[98]</sup>. Insulin regulates brown preadipocyte determination through a neclin-E2F4 interaction that represses PPAR- $\gamma$  transcription *via* a cyclic AMP response element binding protein-dependent pathway<sup>[99]</sup>. Hyperinsulinemia either undergone exogenously (treatment) or endogenously (secretion), is clearly related with weight gain, which is a feature of the MetS. However, several molecules such as TNF- $\alpha$ , leptin, resistin, interact and block multiple steps of insulin signaling and antagonize its effects on adipocytes.

### Growth hormone and insulin like growth factor 1:

Growth hormone (GH) is not only involved in postnatal somatic growth to adulthood, but also has a role in the regulation of metabolic substrates in the control of body composition and body fat distribution, through the combination of lipolytic and anabolic effects<sup>[100]</sup>. In fact, patients with GH deficiency have a smaller number of adipocytes which also has less volume, and these are partially normalized with GH replacement therapy<sup>[101]</sup>. GH is involved in the conversion of preadipocytes into mature adipocytes, and subsequently plays a role in the maturation of adipocytes which makes them sensitive to insulin and IGF-I<sup>[102]</sup>. The effect of GH on adipogenesis seems mainly mediated *via* stimulating Stat5A/5B inducing the transcriptional activity of PPAR in cooperation with C/EBP $\beta$ / $\delta$ <sup>[103]</sup>.

**Thyroid hormones:** Thyroid hormones are involved in the growth and maturation of several organs and tissues during fetal and neonatal development<sup>[104]</sup>. Finally, in

adult life, thyroid hormones regulate energy metabolism and function of organs such as the adipose tissue, liver, heart, skin tissue, muscle or adipose tissue. It has been observed that thyroid function in BAT is mediated by the C/EBPs signaling which induces the expression of thyroid hormone receptor and PGC1 $\alpha$  (PPAR- $\gamma$  coactivator) and deiodinase (D2) activity determines grade of thyroid function “*in situ*”<sup>[105,106]</sup>.

**Glucocorticoids and sexual hormones:** In humans, infusion of hydrocortisone for 6 h increased levels of circulating FFA, and several mechanisms for the lipolysis of glucocorticoids have been observed<sup>[107,108]</sup>. In addition, dexamethasone is involved in the expression of PPAR- $\gamma$  transcription factors and C/EBP $\delta$ , and decreases the expression of pref-1 which is a negative regulator of adipogenesis<sup>[109]</sup>. Therefore, the central obesity phenotype is associated mainly with the consumption of peripheral adipose tissue (lipolysis), and it is observed in human hypercortisolism situations as in Cushing’s syndrome. The adrenal glands and gonads are the main primary source of serum levels of steroid hormones. However, adipose tissue has a full arsenal of enzymes that induce, interconvert, and inactivate peripheral steroid sex hormones<sup>[110]</sup>. The regulation of glucocorticoids levels is critical for the maintenance of homeostasis and the activity in some tissues of 11- $\beta$ -hydroxysteroid dehydrogenase 1 and 2 (11  $\beta$ HSD1 and 2) interconvert the active form of cortisol in other inactive product called cortisone and *vice versa*<sup>[111]</sup>. This enzyme is highly expressed in adipose tissue and an increase in its activity seems involved in an increased level of visceral adipose tissue<sup>[112,113]</sup>. Moreover, the distribution of body fat is characteristically different between men and women; while they are sexually active, resulting in so-called “android or apple” obesity with abdominal fat depot and “gynoid or pear” obesity where fat accumulates predominately in the buttock. However, the actions that sex steroids have on adipogenesis are poorly known. In addition, the main determinants of the action of sex steroids is given by free circulating levels of the hormone in question and the degree of expression in the target organ receptors. The prereceptor tissue-specific metabolism of steroid hormones is also involved in its function. Adipose tissue and preadipocytes have a great activity either cytochrome P450-dependent aromatase and 17 $\beta$ HSD enzymes. Aromatase regulate the rate of formation of androgens into estrogens: Androstenedione to estrone and testosterone to estradiol. Whereas, the 17 $\beta$ HSD is involved in the production of more active forms of testosterone and androstendiona from their weaker precursors, and the rate 17B-HSD/aromatasa in adipose tissue is correlated positively with central adiposity<sup>[110,114]</sup>. Finally, many men with insulin resistance, T2D or MetS present low testosterone concentrations with high or low gonadotropins (25% and 4%, respectively).

**Adipokines:** The developed and mature adipocyte acquires the ability to synthesize and release many

proteins, known generally by Adipokines. These proteins and hormones are involved in energy homeostasis by regulating energy intake and basal metabolism. Therefore, adipose tissue is implicated in the metabolic control of energy substrates such as glucose and lipids, and interacts with several hormonal systems. The molecules produced by adipose tissue act remotely (endocrine) and locally as paracrine and autocrine on stroma and other components of the adipose tissue (blood vessels, inflammatory cells, etc.) and also other tissues such as muscle. All these actions will contribute in the regulation of the different adipose tissue depots, for expanding the size of peripheral adipose tissue or in fat redistribution to other depots.

In obese and insulin resistant patients increased levels of some adipokines (e.g., leptin, resistin) are often observed while others such as adiponectin levels are typically decreased<sup>[115]</sup> (Figure 2).

### Major adipokines

**Leptin:** Leptin is specifically secreted by fat cells whose primary function assigned was to establish an adiposity signal between the amount of developed adipose tissue and satiety centers in the brain completing a negative feedback loop<sup>[116,117]</sup>. People who lose weight following a low calorie diet usually decrease circulating leptin levels. This decrease in leptin appears to mediate reversible decrease in thyroid activity, sympathetic tone, and a decrease in basal energy expenditure<sup>[118]</sup>. Treating leptin deficiency with recombinant leptin reduces food intake and body weight<sup>[119]</sup>. Therefore, in subjects with very low levels of serum leptin, the recombinant leptin treatment also improved several abnormalities including infertility, lipodystrophy and impaired glucose metabolism and impaired immunity<sup>[120-123]</sup>. The expression and release of leptin is controlled by several hormones and factors. Therefore, appears to be stimulated by insulin, glucocorticoids, TNF- $\alpha$ , estrogens, and C/EBPA; by contrast, is decreased by androgens,  $\beta$ 3-adrenergic activity, GH, free fatty acids, and PPAR- $\gamma$  agonist<sup>[124]</sup>. The action of leptin is essential for energy metabolism, but is also involved in the mobilization of lipids from different fat depots and may be related to the protection of some tissues on lipotoxicity syndrome<sup>[125,126]</sup>. Thus, lipid oxidation in cells that have this capacity (mitochondria) could be increased through the signal of leptin and could reduce excessive fatty acids and protect against lipotoxicity in the liver, pancreas, heart, kidney, and muscle tissue (Figures 2 and 3).

**Adiponectin:** Adiponectin is produced specifically in mature adipocytes and RNA abundance is higher in peripheral adipose tissue compared with visceral adipose tissue<sup>[127]</sup>. Adiponectin receptors are G protein-coupled and have high expression in muscle and liver. Adiponectin is involved in lipid oxidation in skeletal muscle and in the liver, and moreover reduce hepatic production glucose load and postprandial hyperglycemic<sup>[128,129]</sup>. An

inverse relationship has been found between plasma adiponectin levels and the development of obesity, insulin resistance and T2D<sup>[130]</sup>. However, conflicting data have been observed between adiponectin levels and the development of cardiovascular disease<sup>[131]</sup>. Adiponectin treatment decreases TNF- $\alpha$  plasma levels and its hepatic production. Adiponectin was able of improving hepatomegaly, steatosis, and alanine aminotransferase levels related with nonalcoholic obese subjects (Figure 2)<sup>[129]</sup>. Finally, adiponectin levels is early decreased in insulin resistance syndrome, even before the onset of obesity, and adiponectin administration improves IS<sup>[132]</sup>.

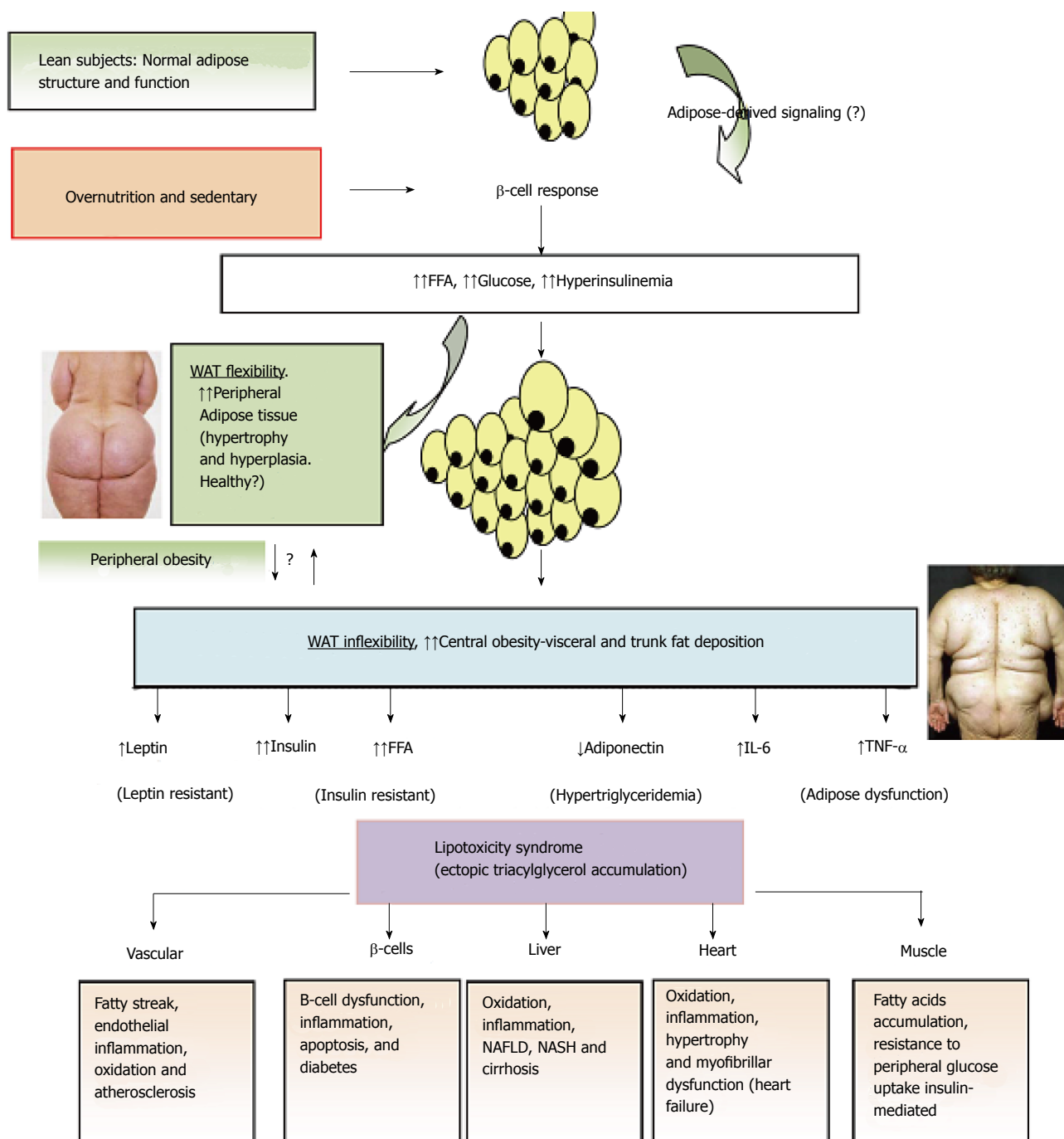
**TNF- $\alpha$ :** TNF- $\alpha$  is a transmembrane protein released mostly by activated macrophages, and also by several other cell types including lymphoid cells, cardiac myocytes, endothelial cells, adipose tissue, etc.<sup>[133-135]</sup> (Figure 2). Therefore, TNF- $\alpha$  is regarded as an adipokine implicated in process of local and systemic inflammation and in proliferation and differentiation of the cells. TNF- $\alpha$  exerts its effects by binding two receptors, TNFR1 (TNF type 1 or CD120a) and TNFR2 (TNF type 2 or CD120b)<sup>[136]</sup>. Both TNF- $\alpha$  gene and its receptors are expressed and modulated in adipocytes and is expressed at higher levels in WAT<sup>[127]</sup>. Some metabolic effects induced by TNF- $\alpha$  implicates it in inhibiting differentiation to mature adipocyte. This in turn leads to insulin resistance, and finally an increase of free fatty acids could result<sup>[137,138]</sup>. In this way, TNF- $\alpha$  treatment decreased the expression of PPAR- $\gamma$  and repressed genes involved in lipid and glucose uptake<sup>[138,139]</sup>.

**IL-6:** IL-6 is secreted by T cells and macrophages involved in the immune response (Figure 2). Smooth muscle cells in blood vessels can also produce IL-6 as a pro-inflammatory cytokine. Finally, IL-6 is synthesized by adipocytes and appears to be associated with elevated levels of CRP and inflammatory states found in obese patients<sup>[140]</sup>. An important part of the total concentration of IL-6 (approximately 1/3) is produced in adipose tissue. However, the expression and release of IL-6 is two to three times higher in visceral adipose tissue compared to peripheral adipose tissue<sup>[127]</sup>. Finally, circulating levels of IL-6 have been found to be directly linked to both obesity and insulin resistance<sup>[141]</sup>. IL-6 inhibits the activity of lipoprotein lipase (LPL) and reduces the differentiation of human preadipocytes, both associated with adipogenesis<sup>[142]</sup>.

### Others main adipokines

**Resistin:** Resistin is a cytokine whose role is not well defined, although firstly was related to obesity, insulin resistance and development of T2D<sup>[143]</sup>.

**Visfatin:** Visfatin is mainly synthesized in the abdominal adipose tissue of humans but not by peripheral adipose tissue, and the first role appeared to have insulin-mimetic actions<sup>[144,145]</sup>. However, the relevance of visfatin



**Figure 3 Adipose tissue expandability and metabolic syndrome.** After a long period of overeating with positive energy balance, associated with increased hormones such as insulin, adipose tissue responds by increasing its storage capacity, which is determined by a number of factors. Individuals with a higher capacity for storing fat, mainly when peripheral WAT is expanded (WAT flexibility), most subjects will remain metabolically normal for a longer period, despite obesity developing. These subjects are observed to be metabolically healthy (MHO). Chronic inflammatory response leads to dysfunctional adipose tissue with increased local and endocrine secretion of acute phase reactants and inflammatory signaling pathways<sup>[265]</sup>. Abnormal cytokine and adipokines production is related to insulin resistance, hyperglycemia, altered lipid profile and cardiovascular diseases<sup>[115,286,287]</sup>. Insulin resistance slowly results from increased accumulation of lipids in other nonadipose tissues such as muscle (lipotoxicity) due to enhanced release of fatty acids from hypertrophic and hyperplastic adipocyte cells. In addition, when adipocytes achieve their maximal storage capacity, they begin to alter their adipokines secretion profile. Therefore, a proinflammatory milieu with elevation in IL-6 and TNF- $\alpha$  and altered adipokines profile, with decreased adiponectin and increased leptin levels, with peripheral leptin resistance, in a dysfunctional adipose system is observed. This suggests that the limitation in storage capacity could be necessary and even precedes the development of metabolic factors. Ectopic lipid accumulation in non-adipocyte cells causes lipotoxicity in these organs and tissues, including inflammation and finally apoptosis. Thus, lipotoxicity in  $\beta$ -cell could decrease beta cell mass (dysfunction of  $\beta$ -cell secretion) and would cause diabetes. Increased fat in liver leads to hepatic steatosis (NAFLD) and steatohepatitis (NASH) and would cause hepatic dysfunction, in the heart would cause myocardial dysfunction, in the endothelial fatty streak would be precursor of generalized arteriosclerosis, etc. At what point the adipose tissue begins to fail is likely to be determined by genetic and epigenetic factors. However, the question is: Can storage capacity in WAT be enhanced to meet an increased demand<sup>[268]</sup>? So far, in human trials, the PPAR- $\gamma$  agonists (TZDs), that remove fat from central deposits toward more favorable peripheral deposits, have been shown to improve lipid profile, insulin-sensitivity, and reduce diabetes and NAFLD<sup>[269]</sup>. WAT: White adipose tissue; MHO: Metabolically healthy obese; IL: Interleukin; TNF- $\alpha$ : Tumor necrosis factor- $\alpha$ ; NAFLD: Non alcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis; PPAR- $\gamma$ : Peroxisome proliferator-activated receptor- $\gamma$ ; TZD: Thiazolidinedione.

in the regulation of glucose metabolism is not clear<sup>[146]</sup>.

**Omentin 1:** Plasma levels and omentin gene expression in visceral adipose are decreased in obesity<sup>[147]</sup>. Omentin 1 is decreased in obese women with polycystic ovary syndrome (PCOS), both glucose and insulin negatively regulate omentin-1 levels *ex vivo* and *in vivo*, and women with PCOS who were treated with metformin increased serum omentin levels<sup>[148,149]</sup>.

### **Effect of fatty acid metabolism and enzymes on adipogenesis**

Fatty acids (FFA) are energy-rich molecules that play a role in metabolism. The excess of calories ingested as fat, protein and carbohydrates, and unspent, are stored as triglycerides (TG; FFA plus glycerol) in mature white adipocytes. They are also an integral part of the cell membrane, conferring functions in fluidity and in the expression of receptors and transporters. In addition, FFA have hormone-like actions and can influence gene expression in preadipocytes, affecting adipogenesis through proliferation and differentiation<sup>[150]</sup>. In humans, food is an important source of FFAs, but biosynthesis could supply most of the fatty acids requirements<sup>[151]</sup>. However, humans are unable to synthesize certain PUFA. Therefore, some precursor in the diet are essentials for two series of PUFA, linoleic acid series ( $\omega$ -6 series) and linolenic acid ( $\omega$ -3 series), that are related with decreased CVD. Today, most diets in the world provide enough  $\omega$ -6 and too little  $\omega$ -3, with an increased ratio  $\omega$ -6:  $\omega$ -3. By contrast, diets with excess saturated fatty acids (and unsaturated trans) have been associated with a significantly increased risk of CVD.

In differentiation and maturation of adipocytes, insulin has a definitive influence increasing the expression and activity of LPL, which is needed for an effective FFA uptake and storage. Adipocytes release and express apo CII and apo CIII by regulating extracellular LPL activity<sup>[152]</sup>. In addition, fatty acid binding proteins (FABPs) are cytoplasmic proteins that carry out intracellular transport of FFA<sup>[153]</sup>. It appears that the expression of fatty acid binding protein-4 (FABP4) is involved in the balance between lipogenesis and lipolysis and in the process of differentiation of preadipocytes. Therefore, it is likely that FABPs serve as a critical link between lipid metabolism, hormone action and cellular function in adipocytes and other cells and thus contribute to systemic energy homeostasis involving glucose metabolism<sup>[154]</sup>.

In humans, "*de novo*" synthesis of straight-chain fatty acids is formed predominantly in the liver where acetyl-CoA is formed from pyruvate, and to a lesser extent in adipose tissue. FFA can be endogenously synthesized from acetyl-CoA and malonyl-CoA precursors through two enzymatic steps, including acetyl-CoA carboxylase (ACC) and FAS. The ACC controls six recurring reactions until production of short fatty acids and then the fatty acids are elongated until 16-carbon palmitic acid is

produced by the action of FAS (Cytosol). Humans can synthesize nearly all fatty acids required from palmitic acid by combining several mechanisms of oxidation and elongation<sup>[155]</sup>. In mammals seven Elovl family enzymes (Elovl1-7) have been identified, and these enzymes are the limitations in control of production by fatty acid elongation<sup>[156]</sup>. The enzyme activity of Elovl3 is transcriptionally regulated by PPAR- $\gamma$ , and in turn the levels of VLCFAs (C18: 1 and C20: 1) produced by the expression of Elovl3 activate PPAR $\gamma$ . Therefore Elovl3-PPAR activity is implicated in the regulation of adipogenesis<sup>[157]</sup>. Saturated fatty acids are amply available from the food by humans, thus FAS enzyme has been shown to have less importance. However, the malonyl-CoA levels are determined by the rate of synthesis by ACC and FAS-mediated catabolic rate, and appear to be an important energy status sensor in the hypothalamus in the metabolic control of body weight<sup>[158]</sup>. Moreover, in the process of differentiation of preadipocytes to mature adipocytes a lower activity of FAS has effects reducing adipose tissue<sup>[159]</sup>. Finally, in the process of synthesis of triglycerides in adipose cells, several enzymes have been observed with an interest in adipogenesis<sup>[160]</sup>. The levels of mRNA and protein of diacylglycerol acyltransferase 1 (DGAT1) increase during the process of differentiation of preadipocytes. DGAT1-deficient mice are resistant to diet-induced obesity associated with a higher energy expenditure. While over-expression of DGAT1 resulting in increased adipose tissue without affecting IS, but increased the secretion of TNF, which interferes with insulin signaling<sup>[161]</sup>.

## **OBESITY AND LIPOTOXICITY SYNDROME**

After absorption in intestine and after synthesis in the liver triglycerides (TG) are packed in specialized lipoproteins [chylomicron and very low density lipoprotein (VLDL)]. They are transported in a network between different locations such as the digestive system, liver, adipose tissue and other tissues. The formation of TG can also be considered a cellular detoxification process by controlling the levels of diacylglycerol and the input and output flows of FFA and acyl-CoA<sup>[162]</sup>. In this regard, droplets containing TG were found in all investigated cells, and even brain tissue has this capacity to form TG. These fat droplets are surrounded by a monolayer of phospholipids hooked by a specific protein called Perilipin (ADRP) which appear to regulate, and are rate limiting factor in its formation, growth and dissolution<sup>[163]</sup>.

Downloading and uptake of free fatty acids in non adipose tissues typically is coupled to its necessity. During periods of fasting and physical exercise should be increased the lipolysis, that is mediated by suppression of plasma insulin and elevation of contrainsulin hormones (glucagon, cortisol, etc.), generating a coupled fuel delivery. Thus, for an optimal mobilization and storage of lipid an efficient adipose tissue is required. By contrast,



after a prolonged overfeeding state, fatty acid load offered may exceed the storage capacity of adipose tissue (inflexibility) (Figure 3). Nuclear receptor PPAR- $\gamma$  is a key gene that regulates adipogenesis and lipid storage, but it appears that is also needed for the control of the lipolysis, dysregulation of which is a prominent characteristic of obesity-induced insulin resistance in humans<sup>[164]</sup>. In addition, the expression of leptin receptor is found in several tissues in the body involving leptin actions in many different sites, including as be a mediator of energy expenditure<sup>[124]</sup>. Leptin secretion rises in parallel with fat expansion in adipocytes and it has been proposed that this prevents lipotoxicity by minimizing ectopic accumulation of lipids into nonadipocytes because leptin induced  $\beta$ -oxidation increasing transcription of PPAR- $\alpha$ . Therefore, excess fatty acids will increase activation of PPAR- $\alpha$  which is a transcription factor of lipolytic enzymes such as carnitine palmitoyl transferase-1 and acyl CoA oxidase. Lipolysis is forced by increasing  $\beta$ -oxidation and uncoupling proteins activity, which corresponds with the observed increase in heat and finally would protect these tissues from the accumulation of fatty acids<sup>[165,166]</sup>. However, although insulin treatment acutely increases leptin levels, it has been observed that patients with insulin resistance syndrome have lower mRNA leptin abundance in adipocytes than IS patients<sup>[115,167]</sup>. In addition, a leptin resistance syndrome in humans for central hypothalamic action has also been found. Finally, this system of chronic increase of  $\beta$ -oxidation can already generate oxidative stress "*per se*" and an inflammatory condition, which can be harmful to these tissues. On the other hand, adiponectin have a key role like insulinsensitizing, anti-inflammatory, anti-apoptotic and pro-angiogenic properties increasing the metabolic flexibility of adipose tissue, *i.e.*, to make adipose tissue more efficient at discharging FFAs when are required and upgrade the rate of FFA re-esterification during the postprandial state<sup>[168]</sup>. Finally, in insulin resistant patients early lower serum adiponectin levels that could not adequately prevent all these processes are observed<sup>[115]</sup>. When these mechanisms are exceeded, an accumulation of fatty acids occurs, and its derived metabolites, which generate lipotoxicity and increased cell death in those tissue not prepared to accumulate this excess of lipids such as muscle,  $\beta$ -cells pancreatic, liver, heart, kidneys, etc.<sup>[126]</sup>.

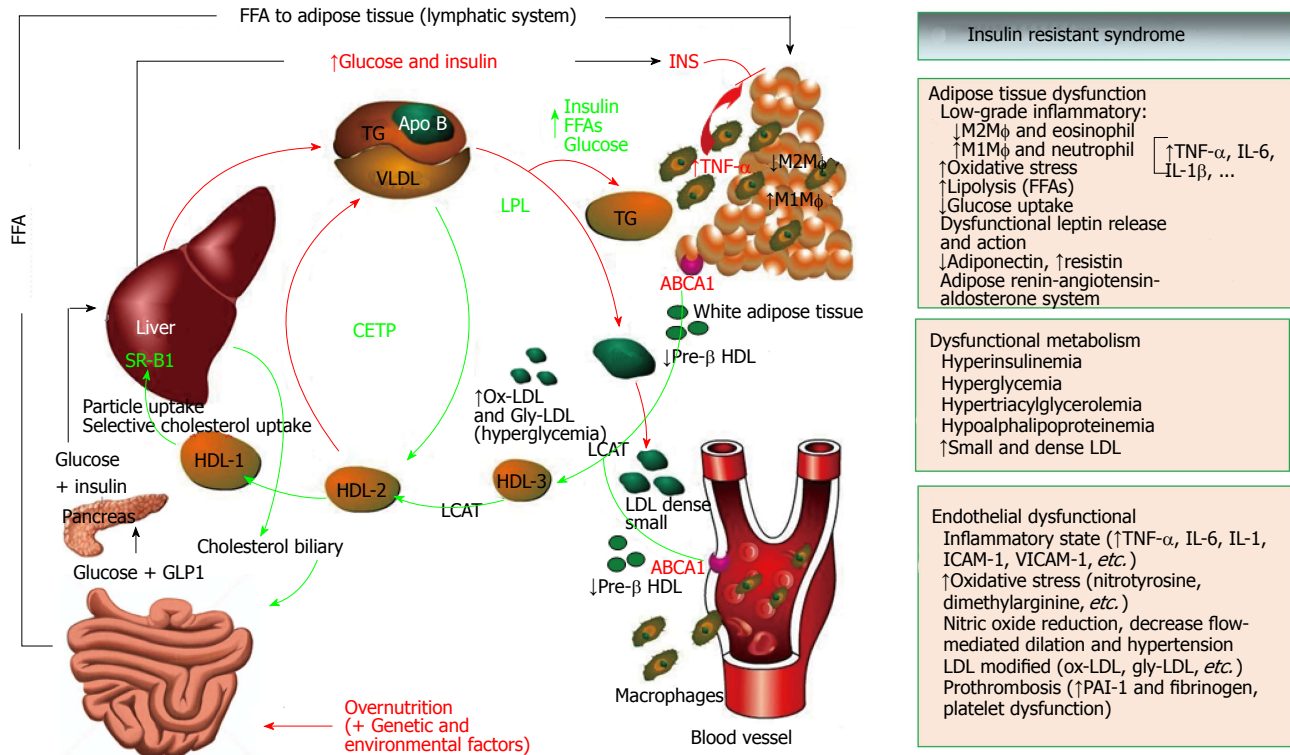
## FROM INSULIN RESISTANCE TO OTHER CARDIOVASCULAR RISK FACTORS

In conditions of overnutrition the adipose tissue (AT) expands to levels of inflexibility (adiposity), and in this state the subject presents a longer postprandial state which leads to hyperinsulinemia, probably the first step in this altered dysfunctional metabolic system (Figure 4). Thus, a lower capacity of disposal and storage of fatty acids associated with an increased lipolysis by AT, and dysfunctional pattern of adipocytokine release (*e.g.*,

decreased adiponectin, and increased leptin, TNF- $\alpha$  and IL-6), may result in inflexibility of AT and indirectly induce redistribution of fat towards undesired and toxic lipids ectopic accumulation. Therefore, when central obesity is slowly being developed, it is observed that hyperinsulinemia and hyperglycemia also progress slowly in postprandial state and later a global hyperglycemia (T2D), hypertriglyceridemia, hypoalbuminemia, hypertension and fatty liver (dysfunctional metabolism) are developed. When a combination of any of these factors cluster together in the same individual the concept of MetS is established<sup>[169]</sup>.

The elevated levels of TG are directed toward white adipose tissue and changes occur in adipocyte size, which leads to changes in its function, and an increase in secretion of TNF- $\alpha$  and Leptin, which stimulates the secretion of monocyte chemotactic protein (MCP-1)<sup>[170]</sup>. This attracts more macrophages to the adipose tissue. Increasing leptin secretion also stimulates macrophage transport to adipose tissue<sup>[171]</sup> and macrophage adhesion to endothelial cells<sup>[172]</sup>. Whatever the stimulus for attracting these macrophages, once present and the recruitment is active, the cytokine production of these macrophages interfere with the normal function of adipocytes (adipose tissue dysfunction)<sup>[173]</sup>. When an inflammatory environment is established in the adipose tissue, the lipid metabolism is altered, initiating postprandial hypertriglyceridemia, because the liver overproduction of VLDL is not removed in time and remains for longer in plasma (postprandial hyperlipidemia). Further, because lipolysis from peripheral adipose tissue is extended, the interstitial content of free fatty acids increases, which can be taken up by the adjacent muscle cells ( $\downarrow$  IS) or again transferred into lipoproteins to the plasma and could be taken up by the liver ( $\uparrow$  VLDL production) and other organs (lipotoxicity). However, not all obese individuals necessarily develop metabolic complications, as some remain insulin sensitive and do not develop fatty liver<sup>[115]</sup>. On top of all these factors, the link between obesity and associated metabolic abnormalities seems to be better related to the topography, anatomical distribution and/or the functional peculiarities of the adipose tissue, a phenomenon which seems to be more relevant in patients with relatively normal weight (Figures 2 and 3).

In obese people elevated triglyceride levels, that are independently associated with an increased risk of cardiovascular disease, are often observed. The liver frees VLDL which are carriers of triglycerides, cholesterol esters and phospholipids, and the hydrolysis of VLDL-TG macromolecule provides cholesterol to peripheral tissues and triglycerides mainly to adipose tissue. The metabolism of triglycerides in adipose tissue is affected by adipokines (leptin and adiponectin) and other factors such as LPL and cholesterol ester transferase protein (CETP)<sup>[174]</sup>. Moreover, the LDL molecules remain longer in plasma, and slowly lose some cholesterol and become small and dense particles, which make these particles more susceptible to changes in oxidation and glycosilation



**Figure 4 Insulin resistant syndrome and lipid metabolism.** When obesity is developing, early abnormalities are observed at this time including hyperinsulinemia and low grade of proinflammatory state (↑ cytokines and PCR-hs), increase liberation of free fatty acids from adipose tissue (↑ lipolysis) and altered release of adipokines (↓ adiponectin, ↑leptin with leptin resistance). In some subjects, fatty liver develops later and consequently affects some functions of the liver. These include an early altered postprandial state (increasing glucose and triglyceride-rich VLDL particles), but finally these findings are observed in fasting state<sup>[289]</sup>. The VLDL particles undergo reduction by LPL and triglycerides are taken up by adipose tissue. The final result is the increase of cholesterol-rich small and dense LDL particles in serum. These LDL particles are highly susceptible to modifications like oxidation and glycation and the result is the increasing levels of ox-LDL, gly-LDL and the generation of antibodies to ox-LDL<sup>[190]</sup>. Finally, modified LDL are phagocytosed by macrophages in endothelial blood vessels and an inflammatory pattern that alters endothelial function initiating arteriosclerosis begins<sup>[177]</sup>. On the other hand, through ABC1 ligand the lipid efflux from peripheral cells to start the reverse transport of cholesterol is mediated. Mature HDL3 are generated from lipid-free apo A1 or lipid-poor pre-β1-HDL as the precursors, and LCAT-mediated esterification of cholesterol generates mature HDL3 and HDL2<sup>[189]</sup>. In T2D insulin-resistant patients, after adequate metabolic control the HDL3 cholesterol and APO A1 levels were increased. These findings were associated with a higher specific binding activity of HDL3 in those patients that showed improved insulin resistance<sup>[190]</sup>. Cholesterol efflux capacity has a strong inverse association with carotid intima-media thickness and was inversely associated with the incidence of cardiovascular events in a population-based cohort<sup>[188,290]</sup>. LCAT-mediated cholesterol esterification generates large spherical HDL2 particles, but large HDL2 can be converted in turn to small HDL3 upon CETP-mediated transfer of CE from HDL to apoB-containing lipoproteins, interfering with reverse cholesterol transport. Finally, SR-B1 mediates the selective uptake of cholesteryl esters from HDL particles into mainly liver and steroidogenic organs<sup>[291]</sup>. VLDL: Very light density lipoprotein; LPL: Lipoprotein lipase; ox-LDL: Oxidized-LDL; gly-LDL: Glycated-LDL; ABC1: ATP-binding cassette transporter 1; LCAT: Lecithin cholesterol acyltransferase; CETP: Cholesteryl ester transfer protein; SR-B1: Scavenger receptor class-B, type I.

(ox-LDL, gly-LDL, etc). The removal and phagocytosis of oxidized and modified forms of LDL cholesterol (LDL-C) by macrophages located in blood vessel walls is a main event in the development of atherosclerosis<sup>[175]</sup>. Under these conditions, also possibly being affected by high insulin levels and increasing macrophage infiltration, which when activated produce proinflammatory cytokines and adhesion molecules (CRP, TNF-α, IL-6, VCAM, ICAM and MCP-1), blood vessels endothelial cells undergoes hypertrophy<sup>[176]</sup>. In early obese T2D patients, even serum ox-LDL levels are influenced by short-term serum glucose variations and flow-mediated endothelium-dependent dilation was decreased and inversely related with increments of circulating ox-LDL levels (endothelial dysfunction)<sup>[177]</sup>. Finally, HDL, which removes surplus cholesterol in peripheral tissues and moves it to the liver either to reuse or excretion, what is recognized as reverse cholesterol transport (RCT), are also lowered by effects

at various points<sup>[178]</sup>. Therefore, elevated triglycerides and decreased HDL-C, also so-called atherosclerotic profile, are considered a risk factor for CVD, independent of LDL-C levels<sup>[174,179-183]</sup>. The RCT begins when small precursors of HDL (nascent Apo AI/HDL, pre-β HDL) accept the cholesterol and phospholipids through interaction with ATP-binding cassette (ABC) transporters ABCA1 and ABCG1<sup>[184]</sup>. ApoA-I is released mainly by the liver and small bowel as lipid-poor apoA-I and nascent phospholipid-rich cholesterol-poor HDL particles. In humans, various mutations in the ABCA1 gene outcome in lowered plasma HDL-C levels and great storage of cholesterol in macrophages located in lymph tissue, and they have an enhanced risk of atherosclerotic events. The liver X receptors LXRα (NR1H3) and LXRβ (NR1H2) have a key role in the control of cholesterol metabolism. Storage intracellular cholesterol levels results in increased cholesterol oxidized forms (oxysterol) which are

endogenous ligands for LXRs; therefore, it is as sensors to keep cholesterol at suitable levels and to equilibrate it in all sites of body<sup>[185]</sup>. The LXR system could intervene in gene expression, controlling the efflux of cholesterol from peripheral cells (macrophages), the elimination of cholesterol from the liver, and the regulation of cholesterol absorption in the small bowel<sup>[186,187]</sup>. Although the efflux of cholesterol from macrophages is a small part of reverse cholesterol flux, it is the most significant component of atheroprotection. Thus, both plasma HDL cholesterol level and the ability to efflux are highly significant indicators of cardiovascular disease status<sup>[188]</sup>. In obesity HDL functions change dramatically during acute and chronic inflammation of adipose tissue, and changes in quality of HDL can contribute to the failure of atheroprotective capacity, and decreased efflux capacity in patients with MetS and diabetes have been shown<sup>[189]</sup>. In addition, after adequate metabolic control of diabetes in T2D insulin resistant patients, the HDL<sub>3</sub> cholesterol and APO A1 levels, directly associated with higher specific binding activity of HDL<sub>3</sub>, were increased<sup>[190]</sup>. Moreover, LCAT (lecithin cholesterol acyl-transferase) enzymes bound to HDL particles play an important role in the change from nascent to mature HDL. LCAT converts free and unesterified cholesterol (form of efflux) in cholesteryl ester, a hydrophobic form of cholesterol (form of transport), that make particles of HDL more spherical and mature. The mature HDL<sub>2</sub> and HDL<sub>3</sub> particles in plasma are constantly remodeled by lipase and interact with other lipoproteins through lipid transfer. This can affect the normal reverse transport of HDL cholesterol to its routes of removal (mainly liver). Therefore, the CETP mediates exchange of HDL cholesteryl ester (CE) with VLDL-triglycerides lipoproteins, and this result in a CE reduction with higher amount of TG in HDL lipoproteins (Figure 4). Thus, in clinical situations of obesity, like insulin resistance and T2D, where VLDL particles are frequently increased (hypertriglyceridemia), HDL cholesterol levels are inversely lowered. In addition, HDL has a variety of anti-atherogenic properties apart from efflux of cholesterol and RCT. It improves endothelial function, inhibits thrombosis and has powerful antioxidant and anti-inflammatory effects.

Last, most patients with features of MetS have increased blood pressure. Several contributing factors such as hyperinsulinemia increases the reabsorption of Na<sup>+</sup> and also activates the sympathetic nervous system. In addition, releasing factors from adipose tissue could stimulate aldosterone secretion independently of angiotensin II, K<sup>+</sup> or ACTH<sup>[191]</sup>. Furthermore, local source of angiotensin II in adipose tissue may also be raised in obese hypertensive subjects establishing the participation of adipose-tissue renin-angiotensin system in insulin resistant syndrome<sup>[192]</sup>.

## FROM OBESITY AND INSULIN RESISTANCE TO METS

MetS was referred to as a group of related metabolic disorders for the first time in 1920 by Kylin. Decades

before of the introduction of measurements with specific methods for insulin, Himsworth (1936) suggests that diabetes could be found two types, what he termed "insulin-sensitive" and "insulin-insensitive" types. Later, Reaven<sup>[193]</sup> (1988) observed that several risk factors (dyslipidemia, hypertension, hyperglycemia) commonly cluster together in insulin resistant subjects (Figure 4). He described it and underscored their clinical importance in their Banting lecture, and he used the name "Syndrome X" but obesity was not including in their definition. Today it is known as "MetS" defined as a "set of metabolic disorders and cardiovascular risk factors, which foresee a high risk of developing diabetes and CVD". The more clinical definition was advanced by Grundy<sup>[194]</sup> in 1999, who described MetS as "a set of metabolic disorders, many of which promoted the development of atherosclerosis and increase the risk of CVD", and were established in the national cholesterol education program's adult treatment panel III report (ATP III) and later updated in 2004<sup>[195]</sup>. It avoids the implication that insulin resistance is the primary or only cause of associated risk factors. In addition, because the presence of abdominal obesity is more highly correlated with the metabolic risk factors, measurement of waist circumference was included as a clinical method to identify patients susceptible to MetS<sup>[196]</sup>. When it is > 102 cm in men and > 88 cm in women it is called abdominal obesity, which is a high risk factor of MetS<sup>[194]</sup>. Other clinical criteria that Grundy established for the diagnosis of MetS were a blood pressure  $\geq 135/85$  mmHg<sup>[197]</sup>, elevated fasting glucose levels  $\geq 110$ <sup>[198]</sup>, triglycerides  $\geq 150$  mg/dL<sup>[199]</sup> and HDL-C < 40 mg/dL for men and < 50 mg/dL for women (Atherogenic dislipemia). When any 3 of the 5 listed characteristics are present, a diagnosis of MetS must be made. A proinflammatory state, clinically observed by elevation of C-reactive protein (CRP-hs), and a prothrombotic state characterized by increased plasma levels of the inhibitor of plasminogen activator (PAI-1) and fibrinogen are also recognized in MetS.

At the same time (1999) the expert committee of the WHO described MetS as a cardiovascular disorder associated with insulin resistance. In order to diagnose MetS according to WHO criteria, insulin resistance should be identified, together with two or more risk factors, with minimal changes of the factors previously described, but including urinary albumin excretion rate  $\geq 20$   $\mu$ g/min or albumin: Creatinine ratio  $\geq 30$  mg/g (microalbuminuria)<sup>[200,201]</sup>.

Last, in order to unify both epidemiologic criteria as clinical, the International Diabetes Federation (IDF) established a set of criteria for diagnosing MetS<sup>[202]</sup>. While the pathogenesis of MetS and each of its components is complex, multifactorial and not well established, either central obesity and insulin resistance or both are recognized as the main causative requirements. Cardiometabolic risk is mainly associated with abdominal obesity because VAT triggers dyslipidemia, insulin resistance and hypertension<sup>[203,204]</sup>. This VAT could be assessed by CT, MRI and DEXA, costly measures and not for everyday use. However WC and WHR may be



used as proxy measures of VAT, as they are correlated with it<sup>[205-207]</sup>. Waist circumference gives a closer approximation of abdominal obesity than BMI, the range being different between ethnic populations with respect to overall adiposity, abdominal obesity and visceral fat<sup>[208-210]</sup>. However, IDF dropped the WHO requirement for insulin resistance but made abdominal obesity necessary as 1 of 5 factors required in the diagnosis. IDF provides the following criteria to define MetS: Central (abdominal) obesity is readily measured using waist circumference and is particularly related with each of the other MetS components, singularly with insulin resistance, and "is a prerequisite risk factor". Abnormality in the distribution of body fat, associated with central obesity and ethnic specific values for waist circumference (BMI  $\geq 30$  kg/m<sup>2</sup>; WC  $\geq 94$  and 80 cm and 102 and 84 cm, respectively for men and women in Europe and United States).

In addition, any two of the following four factors: The atherogenic dyslipidemia with: (1) high levels of triglycerides ( $\geq 150$  mg/dL); (2) reduced cholesterol-HDL ( $< 40$  mg/dL in men and  $< 50$  mg/dL in women), and more precise analysis high level of apolipoprotein B (Apo B) and high number of small and thick LDL particles and small HDL particles<sup>[211]</sup>; (3) Treatment of previously diagnosed hypertension or high blood pressure ( $\geq 130$  mmHg systolic and  $\geq 85$  mmHg diastolic); and (4) The hyperglycemia defined as impaired fasting glucose  $> 100$  mg/dL or previously diagnosed T2D.

Other factors such as genetic profile, physical inactivity, aging, proinflammatory state and hormonal dysregulation could be considered<sup>[202]</sup>.

Therefore, additional metabolic measurements are recommended. Lipodystrophic disorders, either genetic (e.g., Dunnigan familial partial lipodystrophy, Berardinelli-Seip congenital lipodystrophy, etc.) or acquired are almost associated with MetS, and occasionally a genetic study could be considered. Most components of MetS are correlated with a sedentary lifestyle. MetS prevalence and each of its components is directly related with age in most people on the world. Assessment of body fat distribution (DEXA) or central obesity (CT/MRI) or fatty liver content (spectroscopy) could be advised. Proinflammatory state presents an increased levels of CRP, and adipocytes and macrophages release inflammatory cytokines (TNF- $\alpha$ , IL-6), and decrease antiinflammatory adiponectin and increased leptin levels are associated with adipose dysfunction<sup>[212,213]</sup>. Prothrombotic state with increased PAI-1 and fibrinogen<sup>[214]</sup>. Vascular dysregulation (apart of hypertension) could be estimated with endothelial function and presence of microalbuminuria. Insulin resistance with measurements of fasting insulin/proinsulin levels, HOMA-IR<sup>[215]</sup>, by Bergman Minimal Model<sup>[216]</sup>, during oral glucose tolerance test<sup>[217]</sup>, and gold standard from M value from euglycemic-hyperinsulinemic clamp<sup>[218,219]</sup>.

Finally, several organizations have attempted to harmonize criteria for the definition of MetS [International Diabetes Federation Task Force on Epidemiology and

Prevention, National Heart, Lung, and Blood Institute (NHLBI), American Heart Association (AHA), World Heart Federation; International Atherosclerosis Society, and International Association for the Study of Obesity]. They concluded that three abnormal findings out of five would be sufficient to diagnose a person as having MetS. The IDF and AHA/NHLBI agreed that central obesity may not be a prerequisite for diagnosing MetS but could be one of the 5 criteria<sup>[66]</sup>.

## EFFECTS OF NUTRITION ON METS COMPONENTS

The prevalence of MetS based on the ATP criteria rose from 28% in the Third National Health and Nutrition Examination and Survey (NHANES) 1988-1994, to 32% in NHANES 1999-2000. It is estimated that 11% of men and 18% of women between the age of 20-39 have MetS. But, rates increase to 40% in men and 46% in women older than 60 years of age, the frequencies being similar in many developed countries of the world<sup>[220]</sup>. However, at the moment epidemiological and clinical research has released complex and partial information to guide the development of finished nutrition prevention programs. The US Departments of Agriculture and Health and Human Services issued dietary recommendations in the Dietary Guidelines for Americans (DGA), to aid decrease the risk of CVD. This document was also recommended by the AHA (in 2005 and update in 2010) as a dietary proposal to decline the incidence of MetS<sup>[221,222]</sup>. The updated edition of the DGA accentuates about calory density of the nutrient, and recommends a reduced intake of saturated fat and a confined intake of trans fats, but a greater intake of whole grain, variety of fruit and vegetables, and its adherences have been related with a improve in incidence and prevalence of MetS<sup>[223,224]</sup>.

Recently, Scientific Report of the 2015 Dietary Guidelines Advisory Committee (DGAC) also shows that the dietary standard of the majority of the United States people, as well as other developed countries, has a low intake of key food groups that are important sources of shortfall nutrients, including vegetables, fruits, whole grains, and dairy<sup>[225]</sup>. In addition, a higher intake of red and processed meats are shown as harmful compared with a lower intake, and higher ingestion of sugar-sweetened foods and beverages as well as derived of refined grains have been found damaging with moderate to strong evidence. Moreover, the DGAC also found that sodium and saturated fat are being over-consumed by Americans, and probably in many westernized countries as well. However, overweight and obesity rates have continued to increase despite actions to recommend decreasing the percentage of fat in food, suggesting that the actions on obesity are more complex. In addition, the healthy Mediterranean-style diet is one of three diets recommended by DGAC, because variations of this



diet include many components associated with health benefits. Mediterranean diet is part of an ancient culture of nutrition and is being adopted by different peoples and countries. Previously, an elegant study identified the subjects with MetS as a target for dietary therapies to reduce several components of this syndrome. Patients with MetS, received elaborate advice on how to raise daily ingestion of whole grains, vegetables, fruits, nuts, and olive oil; whereas patients in the control group followed a prudent diet. After 2 years, patients that follow the Mediterranean diet had an intake higher in monounsaturated fat, as well as polyunsaturated fat, and fiber and had a decrease ratio of  $\omega$ -6 to  $\omega$ -3 fatty acids. At 2 years of follow-up, patients consuming the Mediterranean diet had significantly reduced serum concentrations of hs-CRP, interleukins, as well as IS, and endothelial function score were improved. Moreover, the Mediterranean diet prevented MetS compared with the control group<sup>[226]</sup>. Last, its beneficial effects have recently been reported among persons at high cardiovascular risk. A Mediterranean diet supplemented with extra-virgin olive oil or nuts reduced the incidence of major cardiovascular events and prevalence of MetS<sup>[38,227]</sup>.

In the prevention and treatment of MetS it has been found that it is not one specific diet, but rather various changes of nutrients in the diet that should be recommended to treat or prevent the onset of each different component of the syndrome.

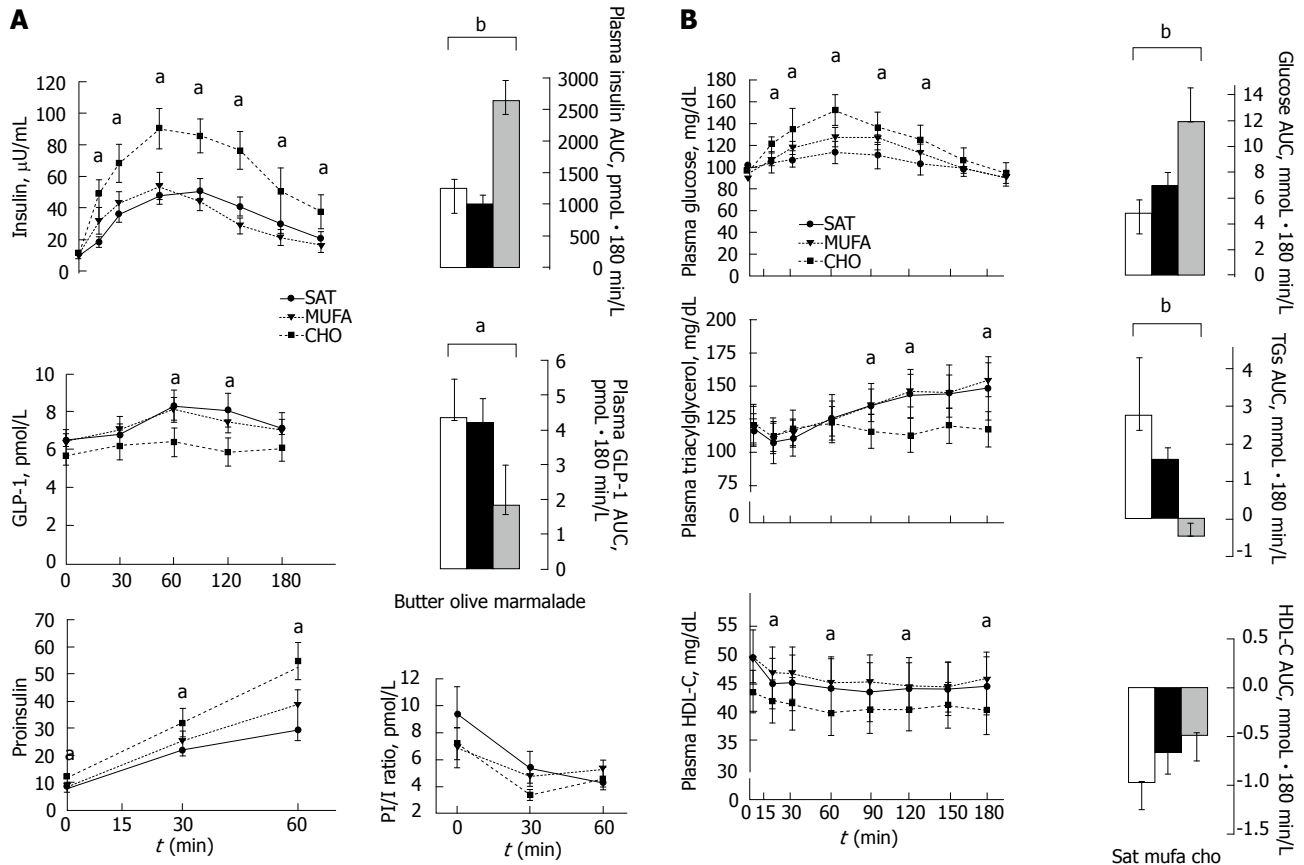
### **Effects of nutrition on obesity**

The first factor to be avoided in the prevention of MetS is obesity, and the percentage of fat in the diet has traditionally been associated with the development of obesity. There is evidence to show that metabolic stressors including energy-dense high-fat diets develop obesity, and probably insulin resistance and MetS<sup>[228-230]</sup>. In overweight subjects, selected on the basis of impaired glucose tolerance, the prevalence of overweight and MetS decreased after two and four years of an extensive life-style intervention which mainly included a reduction of energy and SFA intake and an increase in physical activity<sup>[231,232]</sup>. However, other strong epidemiological evidence has reported contradictory results at this point. An important epidemiological analysis from the European Prospective Investigation into Cancer and Nutrition, which included 519978 participants, found no significant relationship between the amount and type of fat consumed and annual weight gain. Recently, in this cohort it has also been observed that higher SFA consumption was not related with higher ischemic heart disease risk<sup>[230,233]</sup>. But, residual confounding factors, such as cholesterol-lowering therapy and trans fat intake or limited variation in SFA and PUFA intake, may explain these findings. Moreover, in well-conducted intervention studies, in extremely obese subjects with a raised prevalence either diabetes or MetS, a higher weight loss was showed after six months on a carbohydrate-restricted diet than on a fat-restricted

diet, with a relative upgrade in IS and triglyceride levels, even after control for the amount of weight lost<sup>[234]</sup>. Additionally, in a randomized controlled trial to observe weight loss in overweight premenopausal women, where four diets containing a gradual and inverse fat and carbohydrate content were compared, the diet with less carbohydrate content (Atkins) achieved greater weight loss and metabolic success<sup>[235]</sup>. It has also been found that high-protein and low glycemic index (GI) diets are better tolerated than low-protein with high GI. In addition, the low protein with high GI diet was associated with subsequent significant weight regain<sup>[236]</sup>. Further, higher weight loss with low-carbohydrate diets may be associated to the satiating effects of fat and protein content. We have previously found that following the intake of a standard breakfast, the glucagon like peptide-1 (GLP-1) postprandial release was significantly raised in those patients who had eaten an isocaloric olive oil-enriched meal compared to when they had a CHO-rich meal, further supporting the idea that monounsaturated (MUFA) fatty acids may act as secretagogues of GLP-1 (Figure 5)<sup>[237]</sup>. The biological effects of GLP-1 well know include stimulation of glucose-dependent insulin secretion which is lowered until a normal blood glucose level, delay gastric emptying and inhibition of food intake, increases the  $\beta$ -cell proliferation and inhibition of their cell death<sup>[237]</sup>. Finally, epidemiological studies have established an inverse relationship between the consumption of dietary fiber and body weight and waist perimeter<sup>[238,239]</sup>. Therefore, several controlled intervention studies demonstrated that dietary fiber content in the diet is negatively associated with weight gain, and may have a satiating effect and decreases the amount of calories ingested<sup>[240,241]</sup>. Thus, weight loss can be difficult to attain and maintain long-term with interventions of more or less experimental diets. Therefore, important data to reduce and maintain body weight should include the total amount of energy consumed, others characteristics and combinations of the nutrients ingested and the amount and type of physical exercise performed daily. The main interest of research today is to define the potential therapeutic effects of replacing SFA with MUFA or with a low-fat diet on regression of MetS or the effect on the different components of the syndrome.

### **Effects of nutrition on central fat distribution**

It is well established that the type of fat consumed could be more decisive than the total amount of fat consumed when we only look at changes in body composition and distribution of adipose tissue<sup>[242,243]</sup>. It has been proposed that high adiposity and central fat deposit is related to diets with a high ratio of saturated to unsaturated fatty acids<sup>[115]</sup>. In this regard, SFA refers mainly to Myristic (C14), Palmitic (C16) and Stearic (C18) acids; MUFA refers mainly to oleic acid (C18:1n-9) in Western and Mediterranean countries; PUFA refers mainly to linoleic acid (C18:2n-6), a less ratio of alpha-linolenic acid (C18:3n-3) and, in relation of seafood ingested, a



**Figure 5** Mean ( $\pm$  SE) postprandial responses of insulin, proinsulin and glucagon-like peptide-1 levels (A), and glucose, triacylglycerol and high-density lipoprotein cholesterol levels (B), in 11 insulin-resistant subjects to three isocaloric (443 kcal) standard breakfasts. A breakfast rich in carbohydrates, a Mediterranean breakfast enriched with extra-virgin olive oil and standard breakfast high in saturated fat. The incremental AUC was calculated by the formula based on the trapezoid rule with adjustment for baseline concentrations. Repeated measures ANOVA and Tukey's test. <sup>a</sup> $P < 0.05$ ; <sup>b</sup> $P < 0.01$ <sup>[237]</sup>. CHO: High-carbohydrate; MUFA: Monounsaturated fatty acids; AUC: Area under the curve; ANOVA: Analysis of variance.

changeable but lower rate of long chain PUFA such as Arachidonic, eicosapentaenoic (EPA), docosapentaenoic and docosahexaenoic (DHA) acids; and finally TFA reverts to the main trans fatty acids which are isomers of 18:1 trans and are not found in nature and are the result of human processing (e.g., hydrogenation). In this issue, the Nurses' Health Study showed just a weak direct association between whole fat intake and overweight. However, when the proportion of SFA and TFA was higher it showed a strong relationship to obesity, but the consumption of MUFA and PUFA were not associated<sup>[242]</sup>. In addition, MUFA and PUFA fat intake have been associated with healthy effects on body fat distribution and improved some other metabolic disorders, as compared with SFA and TFA intake, while maintaining stable body weight. Therefore, in subjects selected with central obesity, after a short intervention with a low-fat carbohydrate-rich diet, patients grouped according to insulin-resistant state (Matsuda < 4) showed a redistribution of their body fat from peripheral adipose tissue toward central body deposits as compared with isocaloric MUFA-rich diet (Table 1)<sup>[52]</sup>. Moreover, the substitution in the diet of saturated with unsaturated fat, mainly MUFA, resulted in little but consistent loss of body weight, decreased body fat content in limbs

and trunk, while maintaining a high and isocaloric fat content (approximately 40%)<sup>[244]</sup>. Furthermore, the intake of n-3 PUFA, EPA and DHA have been linked to an effect on body weight and body composition. Therefore, higher plasma levels of total n-3 PUFA are related to a decreased BMI and waist and hip circumferences<sup>[245]</sup>. In addition, central fat distribution was negatively related with n-6 PUFA and MUFA in adipose tissue that correlated closely with fatty acids intake in obese patients from a Mediterranean area<sup>[246]</sup>. Recently, the long-term consumption of a LFHCC diet increased fasting FABP4 expression in adipose tissue, while it was reduced by the consumption of LFHCC supplemented with n-3 diet<sup>[247]</sup>. Finally, it was found that conjugated linoleic acids (CLA) produces a reduction on adiposity whereas the lean body mass was not altered or increased, and the waist-hip ratio decreased significantly compared with placebo in adults<sup>[248,249]</sup>. In another study it was found that the rate of body fat lowered in the CLA-treated group, whereas body weight, BMI, and central abdominal diameter were unmodified<sup>[250]</sup>.

### Effects of nutrition on insulin resistance

Insulin resistance is a main characteristic of MetS and is related with other components of the syndrome. The

**Table 1 Composition and body fat distribution after three dietary interventions in insulin-resistant subjects**

	Baseline	High-SAT	High-MUFA	High-CHO	P
EE, (kJ/min)	5.36 ± 0.40	5.49 ± 3.90	5.23 ± 0.37	5.02 ± 0.36	0.3
Anthropometry					
Weight, kg	84.4 ± 5.7	83.2 ± 5.7	83.6 ± 5.8	81.8 ± 6.03	0.3
Total body fat, kg	36.8 ± 4.1	35.0 ± 4.0	35.6 ± 4.0	34.9 ± 4.3	0.1
Lean body mass, kg	47.5 ± 2.5	48.1 ± 2.5	48.9 ± 2.6	46.8 ± 2.1	0.2
Waist to hip ratio	0.99 ± 0.01	0.99 ± 0.01	0.98 ± 0.01	0.98 ± 0.01	0.9
DEXA analysis					
Total body trunk, g	-	37101 ± 2026	38154 ± 1911	39134 ± 2104	0.3
Fatty body trunk, g	-	14313 ± 1362	14842 ± 1437	16459 ± 1653	< 0.05
Total body limb, g	-	36420 ± 3886	36239 ± 3862	32887 ± 3825	0.7
Fat in arm, g	-	7097 ± 1528	7652 ± 1339	7225 ± 1830	0.4
Fat in leg, g	-	8517 ± 1588	8036 ± 1398	7358 ± 1253	< 0.05
Fat trunk:fat leg ratio	-	1.9 ± 0.3	2.1 ± 0.2	2.50 ± 0.2	< 0.05

Data are mean ± SE. P value is analysis of variance for repeated variables. Copyright 2007 American Diabetes Association. *Diabetes Care* 2007; 30: 1717-1723. Reprinted with permission from the American Diabetes Association. EE: Energy expenditure; SAT: Saturated fat; MUFA: Monounsaturated fat; CHO: Carbohydrates rich diets; DEXA: Dual energy X-ray absorptiometry.

KANWU study treated 162 healthy subjects selected at aleatory to eat a controlled, isoenergetic diet for 3 mo containing either a major rate of saturated (SAFA diet) or monounsaturated (MUFA diet) fatty acids. After 3 mo, subjects lowering saturated fatty acid and increasing monounsaturated fatty acid, enhanced IS but had no action on insulin secretion. This favorable effect of different proportion and fat quality on IS was not found in subjects with a fat proportion ingested higher than > 37% of energy eaten<sup>[251]</sup>. In addition, in healthy subjects, it has been shown that isoenergetic substitution of SFA for MUFA or complex carbohydrates (CCHO) improved IS, and other components of MetS such as blood pressure<sup>[252,253]</sup>. In selected subjects with central obesity and insulin-resistance on weight maintenance, a MUFA-rich diet improved IS (HOMA-IR) and fasting proinsulin levels as compared to the CHO-rich diet<sup>[237]</sup>. Finally, in subjects with early diagnosed non alcoholic fatty liver disease (NAFLD), those with more adiposity, higher trunk fat:leg fat ratio (by DEXA) and lower IS, had a higher ratio SAT:MUFA fat intake than insulin sensitive (IS) subjects<sup>[115]</sup>. By contrast, the LIPGENE was the largest human intervention study, pan-European and multicentre, developed to observe the effects and efficacy of changing the type and proportion of dietary fat eaten on IS and other metabolic components that integrate the MetS. This intervention was isoenergetic to avoid the effects of weight modification. At the time, it is partially known the metabolic consequence of adhering to low-SFA diets enriched in MUFA or to LFHCC diets, and whether LC n-3 PUFA can improve the negative effects of a low-fat high-carbohydrate diet in MetS. In conclusion, LC n-3 PUFA supplementation significantly lowered TG and FFA levels in men with MetS. The reduction of dietary SFA had no action on IS, blood pressure, LDL cholesterol levels and factors of inflammation. The LIPGENE study observed that the previous dietary consumed and environment may determine responsiveness to dietary fat modification with respect to IS. More specific dietary

fat modifications may be necessary to significantly improve IS and other components of MetS; perhaps in combination with dietary restriction and weight loss<sup>[254]</sup>. There is evidence that a proportion of fat in the diet in excess of 40% worsens IS, especially when ingested fat is saturated<sup>[251]</sup>. However, recently in this same study those MetS subjects when were selected from the upper HOMA-IR were improved IR, with lowered insulin and HOMA-IR levels after ingestion of the HMUFA and LFHCC n-3 diets. Therefore, specifically insulin-resistant MetS subjects with more metabolic components make a response differently to dietary fat change, being more sensitive to a healthy effect from the exchange of the high SFAs diet by the HMUFA and LFHCC n-3 diets<sup>[255]</sup>.

### Effects of nutrition on glucose metabolism

Other dietary factors that can influence various components of MetS, like postprandial glycemic and insulin levels, triglycerides and HDL-C levels, weight regulation and body composition, as well as fatty liver, are the glycemic load (GL) and the excess of fructose and dietary fiber content of food eaten. On the glycemic index (GI) of a food we identify the area under the curve of blood glucose levels two hours after ingestion of a set amount of CHO where glucose is set to equal 100%. So a low GI food will cause a small rise ( $\leq 55$ ), while a high GI food will trigger a dramatic spike ( $\geq 70$ )<sup>[256]</sup>. Diets higher in fat and a lower content of CHO necessarily have a lower GL and lower GI. Therefore, the beneficial effect of an olive oil enriched diet avoiding simple carbohydrates, e.g., a typical Mediterranean breakfast with wheat bread and olive oil instead of white bread and marmalade, is also found during the postprandial state where lower glucose and insulin AUCs are observed, as compared with CHO-rich diets (Figure 5)<sup>[237]</sup>. By contrast, during an isocaloric low carbohydrate high fat (better MUFA) diet, after absorption the free fatty acids are transported *via* the lymphatic system without stimulating the secretion of insulin, so the fatty acids are carried directly to the

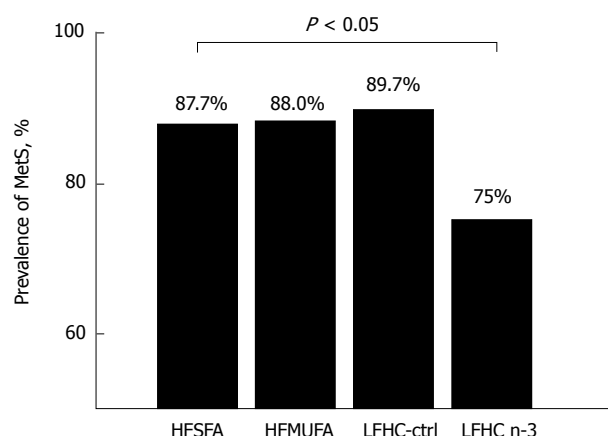
peripheral adipose tissue; thus, postprandial insulin peak and hyperglycemia are reduced<sup>[237]</sup>. These higher postprandial levels of glucose and insulin after eating foods with a high GI or GL may mediate changes on adiposity and central fat redistribution observed in selected insulin-resistant subjects (Table 1)<sup>[52]</sup>. Following intestinal absorption of excess carbohydrates these are transported *via* portal and, after signaling insulin secretion in the pancreas, are deposited in the liver. However, in obese subjects, when the storage limit is exceeded, and through several metabolic pathways, that mainly include the transcription protein carbohydrate response element binding protein, which is activated by a high-carbohydrate diet, the glucose can be used to synthesize fatty acids which are released into plasma as VLDL rich in triglyceride<sup>[257]</sup>. Thus, triglycerides can be captured more widely and again can reach the central depot (Figure 4). Once the function of liver buffer is lost, a state of concomitant hyperglycemia, hyperinsulinemia and hypertriglyceridemia and fatty liver results, due to the consumption of diets high in carbohydrates and high GI. However, conflicting data have been published addressing this concept. It is possible that the type of CHO eaten as well as other macronutrients accompanying these diets could modify and partially explain these discrepant results. Therefore, intervention studies looking at the effects of GI and GL have not had clarifying results. The comparisons of four diets of varying GL on weight loss and cardiovascular risk reduction in a randomized controlled trial was made in 129 overweight and obese young adults<sup>[258]</sup>. The authors concluded that either high-protein or low-GI regimes could have effect on body fat loss, but effects on cardiovascular risk factors are improved by a high-carbohydrate but low-GI diet.

#### **Effects of nutrition on atherogenic dyslipidemia**

The increased levels of triglycerides associated with hypoalphalipoproteinemia, are a feature of insulin resistance and MetS, and increase cardiovascular risk regardless of LDL cholesterol levels. The high insulin levels in MetS constantly target the peripheral adipose tissue and stimulates its hypertrophy, which initiates an aberrant inflammatory condition ( $\uparrow$ M1  $\emptyset$ ) with elevated levels of TNF- $\alpha$  and IL-6 resulting in adipose dysfunction. Therefore the activity of lipoprotein lipase is reduced in AT and the triglyceride clearance is decreased. Adiponectin levels are reduced and the  $\beta$ -oxidation can be lowered by muscles and liver as well as lowering the sensitivity to insulin (Figure 2)<sup>[52,259,260]</sup>. Furthermore, the increase of VLDL ( $\uparrow$ TG) can interact with reverse cholesterol transport by exchanging triglycerides for cholesterol of HDL-C molecules, which eventually can be reduced in plasma. In fact, low HDL-C levels can be considered as one of the earliest signs of a state of insulin resistance. The consumption of a extra-virgin olive-oil-based breakfast by central-obese insulin-resistant subjects lowered postprandial glucose and insulin postprandial excursions, and increased GLP-1 levels as compared with

a isocaloric standard CHO-rich breakfast (Figure 5)<sup>[237]</sup>. In addition, the effects of these dietary interventions on the plasma lipid profile in these insulin-resistant subjects independently of weight loss were also investigated. Serum total cholesterol and Apo B levels tended to decrease after the CHO diets, but a potentially harmful result lowering HDL-C concentrations (approximately 11%) was also observed. By contrast, the consumption of a high MUFA diet was associated with significantly higher HDL-C levels. However, fasting serum triacylglycerol concentrations were not altered by any of the three diets (SAT, MUFA and CHO). These effects could be associated to the fact that body weight was maintained unchanged during the three dietary periods, suggesting that triglycerides levels are mainly related with total body fat<sup>[237]</sup>. By contrast, in the LIPGENE human dietary intervention study, MetS subjects ( $n = 472$ ) from 8 European countries were randomly assigned 4 diets: A HSFA; a HMUFA diet; a LFHCC diet supplemented with long-chain n-3 polyunsaturated fatty acids (1.2 g/d); or a LFHCC diet supplemented with placebo for 12 wk (control). The LFHCC n-3 PUFA diet reduced plasma TG and FFA concentrations, particularly in men<sup>[254]</sup>. Finally, in this study, was made a post hoc analysis, selecting only those patients who had been diagnosed of MetS syndrome (according to NCEP MetS criteria updated by the joint scientific statement harmonizing the MetS criteria) to observe the effect after 12 wk of an isoenergetic dietary fat exchange on final incidence of each component of MetS. In addition, final regression of MetS and each component of MetS post-intervention were also investigated. This study concluded that an isoenergetic LFHCC diet supplemented with LC n-3 PUFA reduced some features of MetS compared with high-fat (HSFA and HMUFA) diets and low-fat diet without LC n-3 PUFA. The prevalence of enlarged waist circumference, hypertension and hypertriacylglycerolemia were reduced after the isoenergetic LFHCC n-3 diet. Thus, the prevalence of MetS fell by 20.5% after LFHCC n-3 diet compared with the HSFA (10.6%), HMUFA (12%) or LFHCC (10.4%) diets (Figure 6)<sup>[261]</sup>. Interestingly, the prevalence of hypertension was reduced after consumption of LFHCC diet supplemented with VLC n-3 PUFA. In a population-based study on food n-3 PUFA intake, an independent inverse relation of total n-3 PUFA intake to systolic and diastolic pressure has previously been shown<sup>[262]</sup>. In addition, the capacity of PUFAs to target the signaling on gene expression of SREBP-dependent, which controls genes implicated in cholesterol metabolism, gives an evidence of the potential effects of fatty acids on gene expression, beyond of purely nutritional<sup>[263]</sup>. Further, it has been observed that n-3 fatty acids but not SAT fatty acids are important activators of PPAR- $\alpha$  implicated in triglycerides reduction. Therefore, because of their capacity to repress inflammatory pathways and control the expression of a great quantity of genes associated to lipid metabolism and adipose tissue, n-3 fatty acids are being using as therapeutic agents in lipids, T2D,





**Figure 6** Prevalence of metabolic syndrome after 12-wk of diet assignment. HFSA is a high fat diet rich in saturated fat and HFMA is a high fat diet rich in monounsaturated fat. LFHC-control is a low-fat, high complex carbohydrate diet and LFHC n-3 is a low-fat, high complex carbohydrate diet supplemented with 1.24 g/d of very long chain n-3 polyunsaturated fatty acid (VLC n-3 PUFA).  $\chi^2$  test,  $P < 0.05$ )<sup>[261]</sup>. MetS: Metabolic syndrome; HFSA: High fat diets rich in saturated fat; HFMA: High fat diets rich in monounsaturated fat; PUFA: Polyunsaturated fatty acids.

steatohepatitis and MetS.

### Effect of fiber on glucose and lipid metabolism

Different fiber content of the diet can influence several components of MetS. The ADA recommends an consumption of dietary fiber of 20 to 35 g per day mainly since of the cholesterol-lowering and glucose-lowering results of soluble fiber. However, more beneficial actions of a higher ingestion of dietary fiber, specially of the soluble form, over the amount advised by the ADA, were reported to get better glycemic control, lowers hyperinsulinemia, and decreases plasma lipid levels in type 2 diabetic patients<sup>[264]</sup>. This should warn us that the intake of complex carbohydrates with high fiber content (e.g., whole bread) have healthier effects compared with refined CHO food popular in modern nutrition. Therefore, maintaining a diet that includes a high intake of fruits, vegetables, and whole grains, a rich sources of dietary fiber, such as a Mediterranean diet, should be strongly emphasized.

### Effect of nutrition on adipokines

We have recently analyzed the repertoire of adipokines in patients diagnosed with fatty liver, a human model of central obesity, much of them with MetS. We confirmed that IR patients had lower serum adiponectin level than IS patients, and a positive correlation between IS index (ISI) and serum adiponectin levels was observed<sup>[115]</sup>. It has been shown that hypoadiponectinemia may play a pathophysiological role in the progression from NAFLD to NASH. Adiponectin exerts a endocrine protective action on liver fat accumulation favoring lipolysis (Figure 2)<sup>[129]</sup>. In addition, we have previously documented a differential postprandial regulation of adiponectin gene expression on peripheral adipose tissue in response to differences in the isocaloric macronutrient composition

of diets. Therefore, after a CHO-rich breakfast a lowered adiponectin mRNA expression levels were found as compared when a MUFA-rich breakfast were eaten<sup>[52]</sup>. The paracrine effects of adiponectin can increase insulin sensitivity by increasing fat  $\beta$ -oxidation and energy expenditure on skeletal muscle<sup>[265]</sup>. Therefore, these actions and a direct adiponectin effect on the ability of adipose tissue to expand it seems play a key role for the regulation in differences in insulin sensitivity and the prevention of central-obesity in responses to different macronutrient composition of diets, in the context of isoenergetic diets and energy balance<sup>[52,115]</sup>. Finally, a recent review on the effects of diet on adiponectin levels summarizes that daily consumption of sea foods or omega-3 supplementation could increase adiponectin concentrations by 14%-60%. In addition, weight loss performed with a low-calorie diet more physical activity raised adiponectin concentrations by 18%-48%. Last, with fiber supplementation were improved adiponectin levels until a 60%-115%<sup>[266]</sup>.

Adiponectin and leptin seem to regulate the deposition of fat in insulin-sensitive tissues by increasing fat oxidation. However, whereas leptin acts on peripheral target and through CNS, adiponectin seems to act mainly on peripheral tissue and liver. Therefore, deposition of fat in the trunk but not in the legs was directly related with increased liver enzyme levels. Fatty liver patients with IR show lower leptin (LEP) mRNA expression in peripheral adipose tissue in comparison with IS patients<sup>[115]</sup>. In these patients we failed to show differences in LEP serum levels between IR and IS patients. Nevertheless, since IR patients were more obese and had higher energy intake in comparison with IS subjects, we speculate that IR patients should have exhibited relatively higher plasma leptin concentrations. This may indicate a dysfunction of adipose tissue in maintaining appropriate levels of leptin to overcome the state of leptin resistance observed in obese subjects particularly where insulin resistance is developed<sup>[115]</sup>. With respect to the response to diets with different macronutrient composition, there is evidence that long-term change in diet (approximately 1 year), including decreased intake of SATs and increased PUFA reduced plasma LEP concentration regardless of changes in fat mass<sup>[267]</sup>. The results of a study conducted in our laboratory, are interesting. The study was on the acute effects of different isocaloric diets during postprandial state and after an insulin treatment on molecular markers characteristically involved in the process of WAT expansion. All patients were previously diagnosed with fatty liver and IR ( $n = 15$ ) and were stabilized for 2 wk with an isocaloric standard diet (National Cholesterol Education Program step 1) for fasting peripheral adipose biopsy. They then randomly eat each one of three isocaloric-specific diets for 4 wk, finally undergoing a postprandial biopsy of adipose tissue after three specific meal test meals; a high saturated fat (SAS), high monounsaturated (MUFA) and low-FAT high-carbohydrates (CHO) (Table 2). The gene-expression array profiles in IR patients showed that acute response

after an isocaloric-specific diet (180 min) (MUFA, SAT and CHO) presented similar postprandial transcripts. Our gene-expression arrays further confirmed that an anabolic stimulus induced by insulin treatment can acutely increase LEP and PPARG gene expression in WAT *per se*. It has been observed that PPARG and insulin are involved in the nutritional regulation of the *fbp27* gene in WAT, which is needed for the most favorable energy storage and performs a key role regulating whole-body energy equilibrium<sup>[268]</sup>. Additionally, this is important because PPARG pharmacologic ligands, such as the thiazolidinediones, increase peripheral AT capacity and decreases liver fat deposition, resulting in a healthier insulin action and liver enzymatic profile<sup>[269]</sup>. However, as with glitazone treatment, insulin therapy is often associated with weight gain due to its lipogenic effects. Therefore, this approach has the potential of initiating a vicious cycle ultimately leading to further obesity and metabolic stress, and eventually to more IR.

### Effect of nutrition on oxidative stress

Finally, we have gathered information about the energy balance of these patients. Our results indicate that in early stages of the disease, changes in REE, RQ, and CHO, fat and protein oxidation could not be differentiated between IR and IS patients. However, the higher waist-to-hip ratio early correlated negatively with CHO oxidation and directly with fat-oxidation, suggesting that central adipose fat distribution could decrease glucose utilization as fuel. In addition, the increase in energy intake in IR patients seemed to be primarily related to their apparent preference for higher saturated fat and refined cereal, sugar and soft drink intake. Several mechanisms have implicated high SAT and sugar diets with the development of fatty liver. This includes its association with higher insulin resistance, as well as an increase in markers associated with endoplasmic reticulum stress, excessive production of reactive oxygen species, leading to inflammatory and proapoptotic responses.

The ability of the adipose tissue to keep in reserve fats in obesity is associated with different cellular actions. Thus, a key function in this issue is improving performance of the endoplasmic reticulum (ER) in the adipocytes. ER is a main organelle that regulates nutrient storage, and the surplus of nutrients increases the amount of altered proteins synthesis which accumulate in the ER. ER stress has been related to many effects of disability cellular that include activation of inflammation and stress networks, closely linked to turn on with by oxidative stress and insulin resistance. The actions of different dietary fat compositions in functions of ER stress on adipose tissue has been investigated in patients with obesity and MetS. In a substudy accomplished within the LIPGENE study, 39 MetS patients were assigned to one of four isocaloric diets: High-SFA (38% E from fat, 16% as SFA), high MUFA (38% E from fat, 20% MUFA), and two low-fat, high-complex carbohydrate (28% E from fat) diets supplemented with 1.24 g/d of long-chain n-3

PUFA or placebo for 12 wk each. This study observed that during the postprandial state, several genes linked to ER stress, such as sXBP-1 and BiP, independent of the fat consumed, in peripheral adipose tissue of patients with MetS, are activated. In addition, after the 12 wk of HSFA diet the expression of *PDIA3* gene was twice higher than after 12 wk of LFHCC n-3 diet. Overall, these data indicate that increase of ER stress in adipose tissue, by amount and different types of fat intake, could play a key role for regulating the capacity of glucose and TG clearance. Thus, ER capacity of AT may modulate metabolic flexibility, initially during postprandial state, accelerating remove of glucose and lipid<sup>[270]</sup>.

Moreover, a high oxidative stress is found in MetS patients, which is showed by a raised activity of NADPH-oxidase and a reduced expression of antioxidant enzymes in the adipose tissue. In patients from the LIPGENE study it was observed that MUFA fat intake decreases oxidative stress as compared with high SAT fat diet by increasing postprandial antioxidant reaction in adipose tissue. Therefore, changing a proportion of SFA by MUFA in the diets could have any beneficial effect to decrease the oxidative stress in MetS patients<sup>[271]</sup>. Last, MetS patients normally present higher inflammatory state in AT, which is increased during postprandial response, which was seen with independence of the fat eaten. We have found that *p65*, *IκBα*, *MCP-1* and *IL-1β* gene transcripts were induced during the postprandial response, also with independence of fat intake. Of note, IL-6 expression was only identify after the postprandial responses<sup>[272]</sup>.

In summary, in patients at risk, achieving and maintaining an ideal body weight, adjusting energy balance between calorie intake and daily regular exercise is essential in preventing the development of MetS, regardless of the distribution of macronutrient energy. However, the composition of macronutrients can have beneficial or harmful effects on several factors of the metabolic profile, and this can be very important in the dietary counseling of patients with MetS.

Therefore, in subjects with early central obesity associated with other components of MetS, the first recommendation would be to reduce calorie intake and ensure daily physical exercise in order to achieve an ideal weight. Secondly, avoid the intake of trans fat, mainly cakes, biscuits, pastries, etc., and moderate the intake of saturated fat, mainly red meat, processed meats and meat sauces. Thirdly, avoid eating simple carbohydrates, such as sugar, soft drinks, and fruit and juices in excess. This will prevent insulin spike, an increment in triglycerides levels, and also improve the reverse transport cholesterol, and probably fatty liver and central obesity. It is preferable to increase the intake of complex carbohydrates with a lower glycemic index such as wholemeal bread and legumes. Moderate intake of white pasta, potatoes, white rice, etc., is permitted, but these should be eaten with plenty of vegetables, thus increasing fiber content will decrease its GI. The fourth recommendation, is to moderate protein intake

**Table 2** Gene expression ARRAYS of peripheral-white adipose tissue in fasting state and its responses to postprandial specific diets and insulin stimulus

	Baseline (n = 8)	P1	High-MUFA (n = 9)	High-SAT (n = 9)	High-CHO (n = 9)	P2	Postinsulin (n = 9)	P3	P4
INSR	6.74 ± 0.11	0.69	6.94 ± 0.06	6.95 ± 0.01	6.98 ± 0.04	0.95	6.99 ± 0.04	0.93	0.10
GCCR	6.25 ± 0.13	0.03	5.92 ± 0.06	5.90 ± 0.07	5.89 ± 0.06	0.95	5.75 ± 0.07	0.93	0.04
BMP7	4.80 ± 0.08	0.69	4.49 ± 0.07	4.45 ± 0.09	4.53 ± 0.11	0.95	4.55 ± 0.09	0.93	0.33
BMP2	6.21 ± 0.10	0.62	5.89 ± 0.09	5.96 ± 0.06	5.92 ± 0.09	0.95	5.89 ± 0.07	0.93	0.12
PPARG	9.06 ± 0.28	0.11	9.64 ± 0.08	9.44 ± 0.10	9.57 ± 0.09	0.95	9.97 ± 0.13	0.14	0.04
ADIPOQ	10.89 ± 0.4	0.69	11.65 ± 0.06	11.33 ± 0.22	11.46 ± 0.11	0.95	11.53 ± 0.16	0.93	0.10
LEP	9.37 ± 0.34	0.60	9.87 ± 0.16	9.64 ± 0.22	9.91 ± 0.18	0.95	10.11 ± 0.14	0.93	0.04
ADRβ3	5.23 ± 0.12	0.69	5.06 ± 0.12	5.01 ± 0.10	5.05 ± 0.17	0.95	5.22 ± 0.11	0.93	0.82
RETN	6.19 ± 0.05	0.02	5.59 ± 0.10	5.63 ± 0.07	5.72 ± 0.09	0.95	5.68 ± 0.08	0.93	0.10
IL-6	4.33 ± 0.12	0.69	4.24 ± 0.10	4.27 ± 0.08	4.43 ± 0.16	0.95	4.26 ± 0.11	0.93	0.60
IL6R	6.50 ± 0.08	0.11	6.83 ± 0.06	6.86 ± 0.06	6.86 ± 0.07	0.95	6.85 ± 0.05	0.93	0.10
TNF-α	5.25 ± 0.05	0.69	5.11 ± 0.07	5.13 ± 0.06	5.26 ± 0.08	0.95	5.08 ± 0.07	0.93	0.10
TNFRSF1A	7.49 ± 0.11	0.69	7.68 ± 0.05	7.79 ± 0.07	7.74 ± 0.06	0.95	7.70 ± 0.06	0.93	0.38
TNFRSF1B	8.19 ± 0.09	0.20	8.51 ± 0.04	8.52 ± 0.04	8.56 ± 0.03	0.95	8.52 ± 0.04	0.93	0.10

Gene-expression profiles of the same patient with NAFLD ( $n = 9$ ) from human peripheral (white) adipose tissue were generated with the use of the Affymetrix U133 Plus 2.0 platform. Gene-chip normalization avoids the use of unstable single housekeeping genes and thus can be technically superior for clinical biomarker studies. Data are mean  $\pm$  SE. P1 value compare baseline in fasting state and postprandial MUFA, SAT and CHO dietary periods with ANOVA repeated measured ( $n = 8$ ). P2 value compares postprandial MUFA, SAT and CHO dietary periods with ANOVA repeated measured ( $n = 9$ ). P3 value compares post-insulin infusion state (180') and postprandial MUFA, SAT and CHO dietary periods with ANOVA repeated measured ( $n = 9$ ). P4 value compares baseline in fasting state and post-insulin infusion state (180') with paired t-test analysis ( $n = 8$ ). P-values for multiple comparisons were adjusted by Hommel's test.  $P < 0.05$  was considered significant. INSR: Insulin receptor; GCCR: Glucagon receptor; BMP: Bone morphogenetic protein; PPARG: Peroxisome proliferator-activated receptor gamma; ADIPOQ: Adiponectin; LEP: Leptin; ADRβ3: Adrenergic β-3 receptor; RETN: Resistin; IL-6: Interleukin-6; IL-6R: Interleukin-6 receptor; TNF-α: Tumor necrosis factor-α; TNFRSF1A: Tumor necrosis factor receptor superfamily, member 1A; TNFRSF1B: Tumor necrosis factor receptor superfamily, member 1B; MUFA: High monounsaturated fatty acid diet; SAT: High saturated fatty acid diet; CHO: Low fat-high carbohydrate diet.

of high biological value associated with polyunsaturated fatty acids  $\omega$ -3, which can be achieved by replacing portions of meat with seafood. Lastly, take abundant and varied vegetables daily in the two main dishes, fresh and steamed, seasoned with moderate portions of extra virgin olive oil and small portions of dried fruits. This will not only ensure vitamin and mineral requirements are met, but will also give the meal a high fiber volume, flatten postprandial blood glucose of carbohydrates eaten, and the dried fruits will ensure that  $\omega$ -6 polyunsaturated needs are met. In addition, olive oil should be used in moderate amounts, not more than 20 cc (approximately 180 kcal) per 1000 calories consumed, thus avoiding their overuse, which can lead to obesity. Olive oil is a healthy fat with obvious improvements in atherogenic lipid profile, and contains polyphenols as well as some fat-soluble vitamins like vitamin E which are natural antioxidants<sup>[273]</sup>. A modest reduction in salt consumption causes significant decreases in blood pressure either hypertensive or normotensive individuals. Thus, the current guidance to decrease salt ingestion to 5-6 g/d should be advised, but a further reduction lower 3 g/d could be required in MetS<sup>[16]</sup>. In addition, moderate ingestion of red wine is related with a inferior prevalence of MetS, as well as with beneficial effects on central adiposity, lipid profile and fasting insulin levels<sup>[274]</sup>. Finally, until more conclusive data, it is essential that 2-3 servings per day of semi-skimmed milk and derivatives, and at least 2-3 eggs per week should be included in the diet. Both nutrients provide proteins of high biological value, provide some needs in essential minerals, and are

reasonably low in fat.

## REFERENCES

- 1 **Bray GA.** Medical consequences of obesity. *J Clin Endocrinol Metab* 2004; **89**: 2583-2589 [PMID: 15181027 DOI: 10.1210/jc.2004-0535]
- 2 **Hill JO, Pagliassotti MJ, Peters JC.** Nongenetic determinants of obesity and fat topography. In: Bouchard C, editor. Genetic determinants of obesity. Boca Raton, FL: CRC Press, 1994: 35-48
- 3 **Kopelman PG.** Obesity as a medical problem. *Nature* 2000; **404**: 635-643 [PMID: 10766250 DOI: 10.1038/35007508]
- 4 **Christakis NA, Fowler JH.** The spread of obesity in a large social network over 32 years. *N Engl J Med* 2007; **357**: 370-379 [PMID: 17652652 DOI: 10.1056/NEJMs0606082]
- 5 **Berghöfer A, Pischon T, Reinhold T, Apovian CM, Sharma AM, Willich SN.** Obesity prevalence from a European perspective: a systematic review. *BMC Public Health* 2008; **8**: 200 [PMID: 18533989 DOI: 10.1186/1471-2458-8-200]
- 6 **World Health Organization.** Obesity and overweight. Available from: URL: <http://www.who.int/dietphysicalactivity/publications/facts/obesity/en/print>
- 7 **Engeland A, Bjørge T, Sjøgaard AJ, Tverdal A.** Body mass index in adolescence in relation to total mortality: 32-year follow-up of 227,000 Norwegian boys and girls. *Am J Epidemiol* 2003; **157**: 517-523 [PMID: 12631541 DOI: 10.1093/aje/kwf219]
- 8 **Flegal KM, Carroll MD, Ogden CL, Johnson CL.** Prevalence and trends in obesity among US adults, 1999-2000. *JAMA* 2002; **288**: 1723-1727 [PMID: 12365955 DOI: 10.1001/jama.288.14.1723]
- 9 **Flegal KM, Carroll MD, Kuczmarski RJ, Johnson CL.** Overweight and obesity in the United States: prevalence and trends, 1960-1994. *Int J Obes Relat Metab Disord* 1998; **22**: 39-47 [PMID: 9481598 DOI: 10.1038/sj.ijo.0800541]
- 10 **Eyre H, Kahn R, Robertson RM.** Preventing cancer, cardiovascular disease, and diabetes: a common agenda for the American Cancer Society, the American Diabetes Association, and the American Heart Association. *CA Cancer J Clin* 2004; **54**: 190-207 [PMID: 15253917]



DOI: 10.3322/canjclin.54.4.190]

- 11 **Pischon T**, Boeing H, Hoffmann K, Bergmann M, Schulze MB, Overvad K, van der Schouw YT, Spencer E, Moons KG, Tjønneland A, Halkjaer J, Jensen MK, Stegger J, Clavel-Chapelon F, Boutron-Ruault MC, Chajes V, Linseisen J, Kaaks R, Trichopoulos A, Trichopoulos D, Bamia C, Sieri S, Palli D, Tumino R, Vineis P, Panico S, Peeters PH, May AM, Bueno-de-Mesquita HB, van Duijnhoven FJ, Hallmans G, Weinehall L, Manjer J, Hedblad B, Lund E, Agudo A, Arriola L, Barricarte A, Navarro C, Martinez C, Quirós JR, Key T, Bingham S, Khaw KT, Boffetta P, Jenab M, Ferrari P, Riboli E. General and abdominal adiposity and risk of death in Europe. *N Engl J Med* 2008; **359**: 2105-2120 [PMID: 19005195 DOI: 10.1056/NEJMoa0801891]
- 12 **Berrington de Gonzalez A**, Hartge P, Cerhan JR, Flint AJ, Hannan L, MacInnis RJ, Moore SC, Tobias GS, Anton-Culver H, Freeman LB, Beeson WL, Clipp SL, English DR, Folsom AR, Freedman DM, Giles G, Hakansson N, Henderson KD, Hoffman-Bolton J, Hoppin JA, Koenig KL, Lee IM, Linet MS, Park Y, Pocobelli G, Schatzkin A, Sesso HD, Weidpass E, Willcox BJ, Wolk A, Zeleniuch-Jacquotte A, Willett WC, Thun MJ. Body-mass index and mortality among 1.46 million white adults. *N Engl J Med* 2010; **363**: 2211-2219 [PMID: 21121834 DOI: 10.1056/NEJMoa1000367]
- 13 **Ezzati M**, Riboli E. Behavioral and dietary risk factors for noncommunicable diseases. *N Engl J Med* 2013; **369**: 954-964 [PMID: 24004122 DOI: 10.1056/NEJMra1203528]
- 14 Food, nutrition, physical activity, and the prevention of cancer: A global perspective. Washington, DC: American Institute for Cancer Research, 2007
- 15 **Mozaffarian D**, Appel LJ, Van Horn L. Components of a cardioprotective diet: new insights. *Circulation* 2011; **123**: 2870-2891 [PMID: 21690503 DOI: 10.1161/CIRCULATIONAHA.110.968735]
- 16 **He FJ**, Li J, Macgregor GA. Effect of longer term modest salt reduction on blood pressure: Cochrane systematic review and meta-analysis of randomised trials. *BMJ* 2013; **346**: f1325 [PMID: 23558162 DOI: 10.1136/bmj.f1325]
- 17 **Sacks FM**, Bray GA, Carey VJ, Smith SR, Ryan DH, Anton SD, McManus K, Champagne CM, Bishop LM, Laranjo N, Leboff MS, Rood JC, de Jonge L, Greenway FL, Loria CM, Obarzanek E, Williamson DA. Comparison of weight-loss diets with different compositions of fat, protein, and carbohydrates. *N Engl J Med* 2009; **360**: 859-873 [PMID: 19246357 DOI: 10.1056/NEJMoa0804748]
- 18 **Mozaffarian D**, Hao T, Rimm EB, Willett WC, Hu FB. Changes in diet and lifestyle and long-term weight gain in women and men. *N Engl J Med* 2011; **364**: 2392-2404 [PMID: 21696306 DOI: 10.1056/NEJMoa1014296]
- 19 **Manson JE**, Willett WC, Stampfer MJ, Colditz GA, Hunter DJ, Hankinson SE, Hennekens CH, Speizer FE. Body weight and mortality among women. *N Engl J Med* 1995; **333**: 677-685 [PMID: 7637744 DOI: 10.1056/NEJM199509143331101]
- 20 **Willett WC**, Manson JE, Stampfer MJ, Colditz GA, Rosner B, Speizer FE, Hennekens CH. Weight, weight change, and coronary heart disease in women. Risk within the 'normal' weight range. *JAMA* 1995; **273**: 461-465 [PMID: 7654270 DOI: 10.1001/jama.1995.03520300035033]
- 21 **Stevens J**, Plankey MW, Williamson DF, Thun MJ, Rust PF, Palesch Y, O'Neil PM. The body mass index-mortality relationship in white and African American women. *Obes Res* 1998; **6**: 268-277 [PMID: 9688103 DOI: 10.1002/j.1550-8528.1998.tb00349.x]
- 22 **Lindsted KD**, Singh PN. Body mass and 26 y risk of mortality among men who never smoked: a re-analysis among men from the Adventist Mortality Study. *Int J Obes Relat Metab Disord* 1998; **22**: 544-548 [PMID: 9665675 DOI: 10.1038/sj.ijo.0800623]
- 23 **Calle EE**, Thun MJ, Petrelli JM, Rodriguez C, Heath CW. Body-mass index and mortality in a prospective cohort of U.S. adults. *N Engl J Med* 1999; **341**: 1097-1105 [PMID: 10511607 DOI: 10.1056/NEJM199910073411501]
- 24 **American Heart Association**. Heart disease and stroke statistics. Dallas, TX: American Heart Association, 2004
- 25 **American Heart Association**. Heart disease and stroke statistics. Dallas, TX: American Heart Association, 2003
- 26 **Suk SH**, Sacco RL, Boden-Albala B, Cheun JF, Pittman JG, Elkind MS, Paik MC; Northern Manhattan Stroke Study. Abdominal obesity and risk of ischemic stroke: the Northern Manhattan Stroke Study. *Stroke* 2003; **34**: 1586-1592 [PMID: 12775882 DOI: 10.1161/01.STR.0000075294.98582.2F]
- 27 **Oster G**, Thompson D, Edelsberg J, Bird AP, Colditz GA. Lifetime health and economic benefits of weight loss among obese persons. *Am J Public Health* 1999; **89**: 1536-1542 [PMID: 10511836 DOI: 10.2105/AJPH.89.10.1536]
- 28 **Mokdad AH**, Ford ES, Bowman BA, Nelson DE, Engelgau MM, Vinicor F, Marks JS. Diabetes trends in the U.S.: 1990-1998. *Diabetes Care* 2000; **23**: 1278-1283 [PMID: 10977060 DOI: 10.2337/diacare.23.9.1278]
- 29 **Laakso M**. Hyperglycemia and cardiovascular disease in type 2 diabetes. *Diabetes* 1999; **48**: 937-942 [PMID: 10331395 DOI: 10.2337/diabetes.48.5.937]
- 30 **Landin K**, Krotkiewski M, Smith U. Importance of obesity for the metabolic abnormalities associated with an abdominal fat distribution. *Metabolism* 1989; **38**: 572-576 [PMID: 2657328 DOI: 10.1016/0026-0495(89)90219-9]
- 31 **Peiris AN**, Mueller RA, Smith GA, Struve MF, Kissebah AH. Splanchnic insulin metabolism in obesity. Influence of body fat distribution. *J Clin Invest* 1986; **78**: 1648-1657 [PMID: 3537010 DOI: 10.1172/JCI112758]
- 32 **Hu FB**, Manson JE, Stampfer MJ, Colditz G, Liu S, Solomon CG, Willett WC. Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. *N Engl J Med* 2001; **345**: 790-797 [PMID: 11556298 DOI: 10.1056/NEJMoa010492]
- 33 **American Diabetes Association**. (4) Foundations of care: education, nutrition, physical activity, smoking cessation, psychosocial care, and immunization. *Diabetes Care* 2015; **38** Suppl: S20-S30 [PMID: 25537702 DOI: 10.2337/dc15-S007]
- 34 **Kayıkçioğlu M**, Özdoğan Ö. [Nutrition and cardiovascular health: 2015 American Dietary Guidelines Advisory Report]. *Türk Kardiyol Dern Ars* 2015; **43**: 667-672 [PMID: 26717326 DOI: 10.5543/tkda.2015.80963]
- 35 **Lew EA**, Garfinkel L. Variations in mortality by weight among 750,000 men and women. *J Chronic Dis* 1979; **32**: 563-576 [PMID: 468958]
- 36 **Calle EE**, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med* 2003; **348**: 1625-1638 [PMID: 12711737 DOI: 10.1056/NEJMoa021423]
- 37 **Willett WC**, Sacks F, Trichopoulos A, Drescher G, Ferro-Luzzi A, Helsing E, Trichopoulos D. Mediterranean diet pyramid: a cultural model for healthy eating. *Am J Clin Nutr* 1995; **61**: 1402S-1406S [PMID: 7754995]
- 38 **Estruch R**, Ros E, Salas-Salvadó J, Covas MI, Corella D, Arós F, Gómez-Gracia E, Ruiz-Gutiérrez V, Fiol M, Lapetra J, Lamuela-Raventós RM, Serra-Majem L, Pintó X, Basora J, Muñoz MA, Sorlí JV, Martínez JA, Martínez-González MA. Primary prevention of cardiovascular disease with a Mediterranean diet. *N Engl J Med* 2013; **368**: 1279-1290 [PMID: 23432189 DOI: 10.1056/NEJMoa1200303]
- 39 **Morris JN**, Heady JA, Raffle PA, Roberts CG, Parks JW. Coronary heart-disease and physical activity of work. *Lancet* 1953; **265**: 1053-1057; contd [PMID: 13110049]
- 40 **Levine JA**, Weisell R, Chevassus S, Martinez CD, Burlingame B, Coward WA. The work burden of women. *Science* 2001; **294**: 812 [PMID: 11679660 DOI: 10.1126/science.1064627]
- 41 **Ezzati M**, Riboli E. Can noncommunicable diseases be prevented? Lessons from studies of populations and individuals. *Science* 2012; **337**: 1482-1487 [PMID: 22997325 DOI: 10.1126/science.1227001]
- 42 **Mozaffarian D**, Afshin A, Benowitz NL, Bittner V, Daniels SR, Franch HA, Jacobs DR, Kraus WE, Kris-Etherton PM, Krummel DA, Popkin BM, Whitsel LP, Zakai NA. Population approaches to improve diet, physical activity, and smoking habits: a scientific statement from the American Heart Association. *Circulation* 2012; **126**: 1514-1563 [PMID: 22907934 DOI: 10.1161/CIR.0b013e318260a20b]
- 43 **Magnusson I**, Rothman DL, Katz LD, Shulman RG, Shulman GI. Increased rate of gluconeogenesis in type II diabetes mellitus. A 13C



- nuclear magnetic resonance study. *J Clin Invest* 1992; **90**: 1323-1327 [PMID: 1401068 DOI: 10.1172/JCI115997]
- 44 Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults--The Evidence Report. National Institutes of Health. *Obes Res* 1998; **6** Suppl 2: 51S-209S [PMID: 9813653]
- 45 Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. *World Health Organ Tech Rep Ser* 1995; **854**: 1-452 [PMID: 8594834]
- 46 Seidell JC, Flegal KM. Assessing obesity: classification and epidemiology. *Br Med Bull* 1997; **53**: 238-252 [PMID: 9246834 DOI: 10.1093/oxfordjournals.bmb.a011611]
- 47 Wang J, Thornton JC, Kolesnik S, Pierson RN. Anthropometry in body composition. An overview. *Ann N Y Acad Sci* 2000; **904**: 317-326 [PMID: 10865763 DOI: 10.1111/j.1749-6632.2000.tb06474.x]
- 48 Ferland M, Després JP, Tremblay A, Pinault S, Nadeau A, Moorjani S, Lupien PJ, Thériault G, Bouchard C. Assessment of adipose tissue distribution by computed axial tomography in obese women: association with body density and anthropometric measurements. *Br J Nutr* 1989; **61**: 139-148 [PMID: 2706220 DOI: 10.1079/BJN19890104]
- 49 Lukaski HC, Bolonchuk WW, Hall CB, Siders WA. Validation of tetrapolar bioelectrical impedance method to assess human body composition. *J Appl Physiol* (1985) 1986; **60**: 1327-1332 [PMID: 3700310]
- 50 Kyle UG, Bosaeus I, De Lorenzo AD, Deurenberg P, Elia M, Gómez JM, Heitmann BL, Kent-Smith L, Melchior JC, Pirllich M, Scharfetter H, Schols AM, Picard C. Bioelectrical impedance analysis--part I: review of principles and methods. *Clin Nutr* 2004; **23**: 1226-1243 [PMID: 15380917 DOI: 10.1016/j.clnu.2004.06.004]
- 51 Taylor RW, Jones IE, Williams SM, Goulding A. Evaluation of waist circumference, waist-to-hip ratio, and the conicity index as screening tools for high trunk fat mass, as measured by dual-energy X-ray absorptiometry, in children aged 3-19 y. *Am J Clin Nutr* 2000; **72**: 490-495 [PMID: 10919946]
- 52 Paniagua JA, Gallego de la Sacristana A, Romero I, Vidal-Puig A, Latre JM, Sanchez E, Perez-Martinez P, Lopez-Miranda J, Perez-Jimenez F. Monounsaturated fat-rich diet prevents central body fat distribution and decreases postprandial adiponectin expression induced by a carbohydrate-rich diet in insulin-resistant subjects. *Diabetes Care* 2007; **30**: 1717-1723 [PMID: 17384344 DOI: 10.2337/dc06-2220]
- 53 Giusti M, Mortara L, Degrandi R, Cecoli F, Mussap M, Rodriguez G, Ferone D, Minuto F. Metabolic and cardiovascular risk in patients with a history of differentiated thyroid carcinoma: A case-controlled cohort study. *Thyroid Res* 2008; **1**: 2 [PMID: 19014658]
- 54 Katherine Zeratsky RD. Normal weight obesity: A hidden health risk? Can you be considered obese if you have a normal body weight? Mayo Clinic - Obesity Expert Answers, 2009
- 55 Deurenberg P, Weststrate JA, Seidell JC. Body mass index as a measure of body fatness: age- and sex-specific prediction formulas. *Br J Nutr* 1991; **65**: 105-114 [PMID: 2043597 DOI: 10.1079/BJN19910073]
- 56 Ashwell M, Cole TJ, Dixon AK. Ratio of waist circumference to height is strong predictor of intra-abdominal fat. *BMJ* 1996; **313**: 559-560 [PMID: 8790002 DOI: 10.1136/bmj.313.7056.559d]
- 57 Cox BD, Whiclow M. Ratio of waist circumference to height is better predictor of death than body mass index. *BMJ* 1996; **313**: 1487 [PMID: 8973270 DOI: 10.1136/bmj.313.7070.1487]
- 58 Hsieh SD, Yoshinaga H. Waist/height ratio as a simple and useful predictor of coronary heart disease risk factors in women. *Intern Med* 1995; **34**: 1147-1152 [PMID: 8929639 DOI: 10.2169/internalmedicine.34.1147]
- 59 Savva SC, Tornaritis M, Savva ME, Kourides Y, Panagi A, Silikiotou N, Georgiou C, Kafatos A. Waist circumference and waist-to-height ratio are better predictors of cardiovascular disease risk factors in children than body mass index. *Int J Obes Relat Metab Disord* 2000; **24**: 1453-1458 [PMID: 11126342 DOI: 10.1038/sj.ijo.0801401]
- 60 Hara M, Saitou E, Iwata F, Okada T, Harada K. Waist-to-height ratio is the best predictor of cardiovascular disease risk factors in Japanese schoolchildren. *J Atheroscler Thromb* 2002; **9**: 127-132 [PMID: 12226553 DOI: 10.5551/jat.9.127]
- 61 Savva SC, Lamnisos D, Kafatos AG. Predicting cardiometabolic risk: waist-to-height ratio or BMI. A meta-analysis. *Diabetes Metab Syndr Obes* 2013; **6**: 403-419 [PMID: 24179379 DOI: 10.2147/DMSO.S34220]
- 62 Vazquez G, Duval S, Jacobs DR, Silventoinen K. Comparison of body mass index, waist circumference, and waist/hip ratio in predicting incident diabetes: a meta-analysis. *Epidemiol Rev* 2007; **29**: 115-128 [PMID: 17494056 DOI: 10.1093/epirev/mxm008]
- 63 Huxley R, Mendis S, Zheleznyakov E, Reddy S, Chan J. Body mass index, waist circumference and waist: hip ratio as predictors of cardiovascular risk--a review of the literature. *Eur J Clin Nutr* 2010; **64**: 16-22 [PMID: 19654593 DOI: 10.1038/ejcn.2009.68]
- 64 Boggs DA, Rosenberg L, Cozier YC, Wise LA, Coogan PF, Ruiz-Narvaez EA, Palmer JR. General and abdominal obesity and risk of death among black women. *N Engl J Med* 2011; **365**: 901-908 [PMID: 21899451 DOI: 10.1056/NEJMoa1104119]
- 65 Lean ME, Han TS, Morrison CE. Waist circumference as a measure for indicating need for weight management. *BMJ* 1995; **311**: 158-161 [PMID: 7613427]
- 66 Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart JC, James WP, Loria CM, Smith SC. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation* 2009; **120**: 1640-1645 [PMID: 19805654 DOI: 10.1161/CIRCULATIONAHA.109.192644]
- 67 Snijder MB, Dekker JM, Visser M, Bouter LM, Stehouwer CD, Yudkin JS, Heine RJ, Nijpels G, Seidell JC. Trunk fat and leg fat have independent and opposite associations with fasting and postload glucose levels: the Hoorn study. *Diabetes Care* 2004; **27**: 372-377 [PMID: 14747216]
- 68 Wajchenberg BL. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. *Endocr Rev* 2000; **21**: 697-738 [PMID: 11133069]
- 69 Hamilton G, Middleton MS, Bydder M, Yokoo T, Schwimmer JB, Kono Y, Patton HM, Lavine JE, Sirlin CB. Effect of PRESS and STEAM sequences on magnetic resonance spectroscopic liver fat quantification. *J Magn Reson Imaging* 2009; **30**: 145-152 [PMID: 19557733 DOI: 10.1002/jmri.21809]
- 70 Kim H, Taksali SE, Dufour S, Befroy D, Goodman TR, Petersen KF, Shulman GI, Caprio S, Constable RT. Comparative MR study of hepatic fat quantification using single-voxel proton spectroscopy, two-point dixon and three-point IDEAL. *Magn Reson Med* 2008; **59**: 521-527 [PMID: 18306404 DOI: 10.1002/mrm.21561]
- 71 Armellini F, Zamboni M, Robbi R, Todesco T, Rigo L, Bergamo-Andreis IA, Bosello O. Total and intra-abdominal fat measurements by ultrasound and computerized tomography. *Int J Obes Relat Metab Disord* 1993; **17**: 209-214 [PMID: 8387970]
- 72 Spiegelman BM, Frank M, Green H. Molecular cloning of mRNA from 3T3 adipocytes. Regulation of mRNA content for glycerophosphate dehydrogenase and other differentiation-dependent proteins during adipocyte development. *J Biol Chem* 1983; **258**: 10083-10089 [PMID: 6411703]
- 73 Mandrup S, Lane MD. Regulating adipogenesis. *J Biol Chem* 1997; **272**: 5367-5370 [PMID: 9102400 DOI: 10.1074/jbc.272.9.5367]
- 74 van Marken Lichtenbelt WD, Vanhommmerig JW, Smulders NM, Drossaerts JM, Kemerink GJ, Bouvy ND, Schrauwen P, Teule GJ. Cold-activated brown adipose tissue in healthy men. *N Engl J Med* 2009; **360**: 1500-1508 [PMID: 19357405 DOI: 10.1056/NEJMoa0808718]
- 75 Virtanen KA, Lidell ME, Orava J, Heglind M, Westergren R, Niemi T, Taittonen M, Laine J, Savisto NJ, Enerbäck S, Nuutila P. Functional brown adipose tissue in healthy adults. *N Engl J Med* 2009; **360**: 1518-1525 [PMID: 19357407 DOI: 10.1056/NEJMoa0808949]
- 76 Cannon B, Nedergaard J. Brown adipose tissue: function and

- physiological significance. *Physiol Rev* 2004; **84**: 277-359 [PMID: 14715917 DOI: 10.1152/physrev.00015.2003]
- 77 **Lean ME**. Brown adipose tissue in humans. *Proc Nutr Soc* 1989; **48**: 243-256 [PMID: 2678120]
- 78 **Cypess AM**, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, Kuo FC, Palmer EL, Tseng YH, Doria A, Kolodny GM, Kahn CR. Identification and importance of brown adipose tissue in adult humans. *N Engl J Med* 2009; **360**: 1509-1517 [PMID: 19357406 DOI: 10.1056/NEJMoa0810780]
- 79 **Schulz TJ**, Huang P, Huang TL, Xue R, McDougall LE, Townsend KL, Cypess AM, Mishina Y, Gussoni E, Tseng YH. Brown-fat paucity due to impaired BMP signalling induces compensatory browning of white fat. *Nature* 2013; **495**: 379-383 [PMID: 23485971 DOI: 10.1038/nature11943]
- 80 **Ouellet V**, Routhier-Labadie A, Bellemare W, Lakhal-Chaieb L, Turcotte E, Carpentier AC, Richard D. Outdoor temperature, age, sex, body mass index, and diabetic status determine the prevalence, mass, and glucose-uptake activity of 18F-FDG-detected BAT in humans. *J Clin Endocrinol Metab* 2011; **96**: 192-199 [PMID: 20943785 DOI: 10.1210/jc.2010-0989]
- 81 **Zingaretti MC**, Crosta F, Vitali A, Guerrieri M, Frontini A, Cannon B, Nedergaard J, Cinti S. The presence of UCP1 demonstrates that metabolically active adipose tissue in the neck of adult humans truly represents brown adipose tissue. *FASEB J* 2009; **23**: 3113-3120 [PMID: 19417078 DOI: 10.1096/fj.09-133546]
- 82 **Cypess AM**, Kahn CR. The role and importance of brown adipose tissue in energy homeostasis. *Curr Opin Pediatr* 2010; **22**: 478-484 [PMID: 20489634 DOI: 10.1097/MOP.0b013e32833a8d6e]
- 83 **Cypess AM**, White AP, Vernochet C, Schulz TJ, Xue R, Sass CA, Huang TL, Roberts-Toler C, Weiner LS, Sze C, Chacko AT, Deschamps LN, Herder LM, Truchan N, Glasgow AL, Holman AR, Gavrilu A, Hasselgren PO, Mori MA, Molla M, Tseng YH. Anatomical localization, gene expression profiling and functional characterization of adult human neck brown fat. *Nat Med* 2013; **19**: 635-639 [PMID: 23603815 DOI: 10.1038/nm.3112]
- 84 **Cinti S**. The adipose organ at a glance. *Dis Model Mech* 2012; **5**: 588-594 [PMID: 22915020 DOI: 10.1242/dmm.009662]
- 85 **Young P**, Arch JR, Ashwell M. Brown adipose tissue in the parametrial fat pad of the mouse. *FEBS Lett* 1984; **167**: 10-14 [PMID: 6698197]
- 86 **Loncar D**. Convertible adipose tissue in mice. *Cell Tissue Res* 1991; **266**: 149-161 [PMID: 1747909]
- 87 **Sung HK**, Doh KO, Son JE, Park JG, Bae Y, Choi S, Nelson SM, Cowling R, Nagy K, Michael IP, Koh GY, Adamson SL, Pawson T, Nagy A. Adipose vascular endothelial growth factor regulates metabolic homeostasis through angiogenesis. *Cell Metab* 2013; **17**: 61-72 [PMID: 23312284 DOI: 10.1016/j.cmet.2012.12.010S1550-4131(12)00501-3]
- 88 **Spalding KL**, Arner E, Westermark PO, Bernard S, Buchholz BA, Bergmann O, Blomqvist L, Hoffstedt J, Näslund E, Britton T, Concha H, Hassan M, Rydén M, Frisén J, Arner P. Dynamics of fat cell turnover in humans. *Nature* 2008; **453**: 783-787 [PMID: 18454136 DOI: 10.1038/nature06902]
- 89 **Kim JB**, Sarraf P, Wright M, Yao KM, Mueller E, Solanes G, Lowell BB, Spiegelman BM. Nutritional and insulin regulation of fatty acid synthetase and leptin gene expression through ADD1/SREBP1. *J Clin Invest* 1998; **101**: 1-9 [PMID: 9421459 DOI: 10.1172/JCI1411]
- 90 **Kim JB**, Wright HM, Wright M, Spiegelman BM. ADD1/SREBP1 activates PPARgamma through the production of endogenous ligand. *Proc Natl Acad Sci USA* 1998; **95**: 4333-4337 [PMID: 9539737]
- 91 **Maroto M**, Bone RA, Dale JK. Somatogenesis. *Development* 2012; **139**: 2453-2456 [PMID: 22736241 DOI: 10.1242/dev.069310]
- 92 **Seale P**, Bjork B, Yang W, Kajimura S, Chin S, Kuang S, Scimè A, Devarakonda S, Conroe HM, Erdjument-Bromage H, Tempst P, Rudnicki MA, Beier DR, Spiegelman BM. PRDM16 controls a brown fat/skeletal muscle switch. *Nature* 2008; **454**: 961-967 [PMID: 18719582 DOI: 10.1038/nature07182]
- 93 **Kajimura S**, Seale P, Tomaru T, Erdjument-Bromage H, Cooper MP, Ruas JL, Chin S, Tempst P, Lazar MA, Spiegelman BM. Regulation of the brown and white fat gene programs through a PRDM16/CtBP transcriptional complex. *Genes Dev* 2008; **22**: 1397-1409 [PMID: 18483224 DOI: 10.1101/gad.1666108]
- 94 **Tseng YH**, Kokkotou E, Schulz TJ, Huang TL, Winnay JN, Taniguchi CM, Tran TT, Suzuki R, Espinoza DO, Yamamoto Y, Ahrens MJ, Dudley AT, Norris AW, Kulkarni RN, Kahn CR. New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. *Nature* 2008; **454**: 1000-1004 [PMID: 18719589 DOI: 10.1038/nature07221]
- 95 **Shan T**, Liang X, Bi P, Zhang P, Liu W, Kuang S. Distinct populations of adipogenic and myogenic Myf5-lineage progenitors in white adipose tissues. *J Lipid Res* 2013; **54**: 2214-2224 [PMID: 23740968 DOI: 10.1194/jlr.M038711]
- 96 **Waldén TB**, Hansen IR, Timmons JA, Cannon B, Nedergaard J. Recruited vs. nonrecruited molecular signatures of brown, "brite," and white adipose tissues. *Am J Physiol Endocrinol Metab* 2012; **302**: E19-E31 [PMID: 21828341 DOI: 10.1152/ajpendo.00249.2011]
- 97 **Rosenwald M**, Perdikari A, Rülcke T, Wolfrum C. Bi-directional interconversion of brite and white adipocytes. *Nat Cell Biol* 2013; **15**: 659-667 [PMID: 23624403 DOI: 10.1038/ncb2740]
- 98 **Accili D**, Taylor SI. Targeted inactivation of the insulin receptor gene in mouse 3T3-L1 fibroblasts via homologous recombination. *Proc Natl Acad Sci USA* 1991; **88**: 4708-4712 [PMID: 2052553]
- 99 **Tseng YH**, Butte AJ, Kokkotou E, Yehoor VK, Taniguchi CM, Kriauciunas KM, Cypess AM, Niinobe M, Yoshikawa K, Patti ME, Kahn CR. Prediction of preadipocyte differentiation by gene expression reveals role of insulin receptor substrates and necln. *Nat Cell Biol* 2005; **7**: 601-611 [PMID: 15895078 DOI: 10.1038/ncb1259]
- 100 **Nam SY**, Lobie PE. The mechanism of effect of growth hormone on preadipocyte and adipocyte function. *Obes Rev* 2000; **1**: 73-86 [PMID: 12119989]
- 101 **Salomon F**, Cuneo RC, Hesp R, Sönksen PH. The effects of treatment with recombinant human growth hormone on body composition and metabolism in adults with growth hormone deficiency. *N Engl J Med* 1989; **321**: 1797-1803 [PMID: 2687691 DOI: 10.1056/NEJM198912283212605]
- 102 **Wabitsch M**, Hauner H, Heinze E, Teller WM. The role of growth hormone/insulin-like growth factors in adipocyte differentiation. *Metabolism* 1995; **44**: 45-49 [PMID: 7476311]
- 103 **Kawai M**, Namba N, Mushiaki S, Etani Y, Nishimura R, Makishima M, Ozono K. Growth hormone stimulates adipogenesis of 3T3-L1 cells through activation of the Stat5A/5B-PPARgamma pathway. *J Mol Endocrinol* 2007; **38**: 19-34 [PMID: 17242167 DOI: 10.1677/jme.1.02154]
- 104 **Morreale de Escobar G**, Obregon MJ, Escobar del Rey F. Role of thyroid hormone during early brain development. *Eur J Endocrinol* 2004; **151** Suppl 3: U25-U37 [PMID: 15554884]
- 105 **Park EA**, Song S, Olive M, Roesler WJ. CCAAT-enhancer-binding protein alpha (C/EBP alpha) is required for the thyroid hormone but not the retinoic acid induction of phosphoenolpyruvate carboxykinase (PEPCK) gene transcription. *Biochem J* 1997; **322** (Pt 1): 343-349 [PMID: 9078282]
- 106 **Carmona MC**, Iglesias R, Obregón MJ, Darlington GJ, Villarroya F, Giral M. Mitochondrial biogenesis and thyroid status maturation in brown fat require CCAAT/enhancer-binding protein alpha. *J Biol Chem* 2002; **277**: 21489-21498 [PMID: 11940593 DOI: 10.1074/jbc.M201710200]
- 107 **Divertie GD**, Jensen MD, Miles JM. Stimulation of lipolysis in humans by physiological hypercortisolemia. *Diabetes* 1991; **40**: 1228-1232 [PMID: 1936585]
- 108 **Xu C**, He J, Jiang H, Zu L, Zhai W, Pu S, Xu G. Direct effect of glucocorticoids on lipolysis in adipocytes. *Mol Endocrinol* 2009; **23**: 1161-1170 [PMID: 19443609 DOI: 10.1210/me.2008-0464]
- 109 **Smas CM**, Chen L, Zhao L, Latasa MJ, Sul HS. Transcriptional repression of pref-1 by glucocorticoids promotes 3T3-L1 adipocyte differentiation. *J Biol Chem* 1999; **274**: 12632-12641 [PMID: 10212243]
- 110 **Bélanger C**, Luu-The V, Dupont P, Tchernof A. Adipose tissue intracrinology: potential importance of local androgen/estrogen metabolism in the regulation of adiposity. *Horm Metab Res* 2002; **34**:

- 737-745 [PMID: 12660892 DOI: 10.1055/s-2002-38265]
- 111 **Pereira CD**, Azevedo I, Monteiro R, Martins MJ. 11 $\beta$ -Hydroxysteroid dehydrogenase type 1: relevance of its modulation in the pathophysiology of obesity, the metabolic syndrome and type 2 diabetes mellitus. *Diabetes Obes Metab* 2012; **14**: 869-881 [PMID: 22321826 DOI: 10.1111/j.1463-1326.2012.01582.x]
- 112 **Bujalska IJ**, Walker EA, Tomlinson JW, Hewison M, Stewart PM. 11 $\beta$ -Hydroxysteroid dehydrogenase type 1 in differentiating omental human preadipocytes: from de-activation to generation of cortisol. *Endocr Res* 2002; **28**: 449-461 [PMID: 12530648]
- 113 **Stewart PM**, Tomlinson JW. Cortisol, 11 beta-hydroxysteroid dehydrogenase type 1 and central obesity. *Trends Endocrinol Metab* 2002; **13**: 94-96 [PMID: 11893517]
- 114 **Meseguer A**, Puche C, Cabero A. Sex steroid biosynthesis in white adipose tissue. *Horm Metab Res* 2002; **34**: 731-736 [PMID: 12660891 DOI: 10.1055/s-2002-38249]
- 115 **Paniagua JA**, Escandell-Morales JM, Gil-Contreras D, Berral de la Rosa FJ, Romero-Jimenez M, Gómez-Urbano A, Sanchez-Lopez A, Bellido E, Poyato A, Calatayud B, Vidal-Puig AJ. Central obesity and altered peripheral adipose tissue gene expression characterize the NAFLD patient with insulin resistance: Role of nutrition and insulin challenge. *Nutrition* 2014; **30**: 177-185 [PMID: 24377452 DOI: 10.1016/j.nut.2013.07.017]
- 116 **Friedman JM**, Halaas JL. Leptin and the regulation of body weight in mammals. *Nature* 1998; **395**: 763-770 [PMID: 9796811 DOI: 10.1038/27376]
- 117 **Friedman JM**. Leptin, leptin receptors, and the control of body weight. *Nutr Rev* 1998; **56**: s38-46; discussion s54-75 [PMID: 9564176]
- 118 **Chan JL**, Heist K, DePaoli AM, Veldhuis JD, Mantzoros CS. The role of falling leptin levels in the neuroendocrine and metabolic adaptation to short-term starvation in healthy men. *J Clin Invest* 2003; **111**: 1409-1421 [PMID: 12727933 DOI: 10.1172/JCI17490]
- 119 **Farooqi IS**, Jebb SA, Langmack G, Lawrence E, Cheetham CH, Prentice AM, Hughes IA, McCamish MA, O'Rahilly S. Effects of recombinant leptin therapy in a child with congenital leptin deficiency. *N Engl J Med* 1999; **341**: 879-884 [PMID: 10486419 DOI: 10.1056/NEJM199909163411204]
- 120 **Welt CK**, Chan JL, Bullen J, Murphy R, Smith P, DePaoli AM, Karalis A, Mantzoros CS. Recombinant human leptin in women with hypothalamic amenorrhea. *N Engl J Med* 2004; **351**: 987-997 [PMID: 15342807 DOI: 10.1056/NEJMoa040388]
- 121 **Petersen KF**, Oral EA, Dufour S, Befroy D, Ariyan C, Yu C, Cline GW, DePaoli AM, Taylor SI, Gorden P, Shulman GI. Leptin reverses insulin resistance and hepatic steatosis in patients with severe lipodystrophy. *J Clin Invest* 2002; **109**: 1345-1350 [PMID: 12021250 DOI: 10.1172/JCI15001]
- 122 **Oral EA**, Simha V, Ruiz E, Andewelt A, Premkumar A, Snell P, Wagner AJ, DePaoli AM, Reitman ML, Taylor SI, Gorden P, Garg A. Leptin-replacement therapy for lipodystrophy. *N Engl J Med* 2002; **346**: 570-578 [PMID: 11856796 DOI: 10.1056/NEJMoa012437]
- 123 **Lord GM**, Matarese G, Howard JK, Baker RJ, Bloom SR, Lechler RI. Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. *Nature* 1998; **394**: 897-901 [PMID: 9732873 DOI: 10.1038/29795]
- 124 **Margetic S**, Gazzola C, Pegg GG, Hill RA. Leptin: a review of its peripheral actions and interactions. *Int J Obes Relat Metab Disord* 2002; **26**: 1407-1433 [PMID: 12439643 DOI: 10.1038/sj.ijo.0802142]
- 125 **Unger RH**, Zhou YT. Lipotoxicity of beta-cells in obesity and in other causes of fatty acid spillover. *Diabetes* 2001; **50** Suppl 1: S118-S121 [PMID: 11272168]
- 126 **Unger RH**, Orci L. Diseases of liporegulation: new perspective on obesity and related disorders. *FASEB J* 2001; **15**: 312-321 [PMID: 11156947 DOI: 10.1096/fj.00-0590]
- 127 **Fain JN**, Madan AK, Hiler ML, Cheema P, Bahouth SW. Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans. *Endocrinology* 2004; **145**: 2273-2282 [PMID: 14726444 DOI: 10.1210/en.2003-1336]
- 128 **Fruebis J**, Tsao TS, Javorschi S, Ebbets-Reed D, Erickson MR, Yen FT, Bihain BE, Lodish HF. Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. *Proc Natl Acad Sci USA* 2001; **98**: 2005-2010 [PMID: 11172066 DOI: 10.1073/pnas.041591798]
- 129 **Xu A**, Wang Y, Keshaw H, Xu LY, Lam KS, Cooper GJ. The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. *J Clin Invest* 2003; **112**: 91-100 [PMID: 12840063 DOI: 10.1172/JCI17797]
- 130 **Lau DC**, Dhillon B, Yan H, Szmitko PE, Verma S. Adipokines: molecular links between obesity and atherosclerosis. *Am J Physiol Heart Circ Physiol* 2005; **288**: H2031-H2041 [PMID: 15653761 DOI: 10.1152/ajpheart.01058.2004]
- 131 **Kanhai DA**, Kranendonk ME, Uiterwaal CS, van der Graaf Y, Kappelle LJ, Visseren FL. Adiponectin and incident coronary heart disease and stroke. A systematic review and meta-analysis of prospective studies. *Obes Rev* 2013; **14**: 555-567 [PMID: 23495931 DOI: 10.1111/obr.12027]
- 132 **Hotta K**, Funahashi T, Bodkin NL, Ortmeier HK, Arita Y, Hansen BC, Matsuzawa Y. Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys. *Diabetes* 2001; **50**: 1126-1133 [PMID: 11334417 DOI: 10.2337/diabetes.50.5.1126]
- 133 **Carswell EA**, Old LJ, Kassel RL, Green S, Fiore N, Williamson B. An endotoxin-induced serum factor that causes necrosis of tumors. *Proc Natl Acad Sci USA* 1975; **72**: 3666-3670 [PMID: 1103152 DOI: 10.1073/pnas.72.9.3666]
- 134 **Pennica D**, Nedwin GE, Hayflick JS, Seeburg PH, Derynck R, Palladino MA, Kohr WJ, Aggarwal BB, Goeddel DV. Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxin. *Nature* 1984; **312**: 724-729 [PMID: 6392892 DOI: 10.1038/312724a0]
- 135 **Locksley RM**, Killeen N, Lenardo MJ. The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell* 2001; **104**: 487-501 [PMID: 11239407 DOI: 10.1016/S0092-8674(01)00237-9]
- 136 **Chen G**, Goeddel DV. TNF-R1 signaling: a beautiful pathway. *Science* 2002; **296**: 1634-1635 [PMID: 12040173 DOI: 10.1126/science.1071924]
- 137 **Ruan H**, Lodish HF. Insulin resistance in adipose tissue: direct and indirect effects of tumor necrosis factor- $\alpha$ . *Cytokine Growth Factor Rev* 2003; **14**: 447-455 [PMID: 12948526 DOI: 10.1016/S1359-6101(03)00052-2]
- 138 **Xing H**, Northrop JP, Grove JR, Kilpatrick KE, Su JL, Ringold GM. TNF  $\alpha$ -mediated inhibition and reversal of adipocyte differentiation is accompanied by suppressed expression of PPAR $\gamma$  without effects on PPAR $\gamma$  expression. *Endocrinology* 1997; **138**: 2776-2783 [PMID: 9202217]
- 139 **Ruan H**, Hacohen N, Golub TR, Van Parijs L, Lodish HF. Tumor necrosis factor- $\alpha$  suppresses adipocyte-specific genes and activates expression of preadipocyte genes in 3T3-L1 adipocytes: nuclear factor- $\kappa$ B activation by TNF- $\alpha$  is obligatory. *Diabetes* 2002; **51**: 1319-1336 [PMID: 11978627 DOI: 10.2337/diabetes.51.5.1319]
- 140 **Bastard JP**, Jardel C, Delattre J, Hainque B, Bruckert E, Oberlin F. Evidence for a link between adipose tissue interleukin-6 content and serum C-reactive protein concentrations in obese subjects. *Circulation* 1999; **99**: 2221-2222 [PMID: 10217702]
- 141 **Fernández-Real JM**, Ricart W. Insulin resistance and chronic cardiovascular inflammatory syndrome. *Endocr Rev* 2003; **24**: 278-301 [PMID: 12788800 DOI: 10.1210/er.2002-0010]
- 142 **Wang B**, Jenkins JR, Trayhurn P. Expression and secretion of inflammation-related adipokines by human adipocytes differentiated in culture: integrated response to TNF- $\alpha$ . *Am J Physiol Endocrinol Metab* 2005; **288**: E731-E740 [PMID: 15562246 DOI: 10.1152/ajpendo.00475.2004]
- 143 **Steppan CM**, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, Lazar MA. The hormone resistin links obesity to diabetes. *Nature* 2001; **409**: 307-312 [PMID: 11201732]



- DOI: 10.1038/35053000]
- 144 **Fukuhara A**, Matsuda M, Nishizawa M, Segawa K, Tanaka M, Kishimoto K, Matsuki Y, Murakami M, Ichisaka T, Murakami H, Watanabe E, Takagi T, Akiyoshi M, Ohtsubo T, Kihara S, Yamashita S, Makishima M, Funahashi T, Yamanaka S, Hiramatsu R, Matsuzawa Y, Shimomura I. Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. *Science* 2005; **307**: 426-430 [PMID: 15604363 DOI: 10.1126/science.1097243]
  - 145 **Fukuhara A**, Matsuda M, Nishizawa M, Segawa K, Tanaka M, Kishimoto K, Matsuki Y, Murakami M, Ichisaka T, Murakami H, Watanabe E, Takagi T, Akiyoshi M, Ohtsubo T, Kihara S, Yamashita S, Makishima M, Funahashi T, Yamanaka S, Hiramatsu R, Matsuzawa Y, Shimomura I. Retraction. *Science* 2007; **318**: 565 [PMID: 17962537 DOI: 10.1126/science.318.5850.565b]
  - 146 **Arner P**. Visfatin--a true or false trail to type 2 diabetes mellitus. *J Clin Endocrinol Metab* 2006; **91**: 28-30 [PMID: 16401830 DOI: 10.1210/jc.2005-2391]
  - 147 **de Souza Batista CM**, Yang RZ, Lee MJ, Glynn NM, Yu DZ, Pray J, Ndubizu K, Patil S, Schwartz A, Kligman M, Fried SK, Gong DW, Shuldiner AR, Pollin TI, McLenithan JC. Omentin plasma levels and gene expression are decreased in obesity. *Diabetes* 2007; **56**: 1655-1661 [PMID: 17329619 DOI: 10.2337/db06-1506]
  - 148 **Tan BK**, Adya R, Farhatullah S, Lewandowski KC, O'Hare P, Lehnert H, Randeve HS. Omentin-1, a novel adipokine, is decreased in overweight insulin-resistant women with polycystic ovary syndrome: ex vivo and in vivo regulation of omentin-1 by insulin and glucose. *Diabetes* 2008; **57**: 801-808 [PMID: 18174521 DOI: 10.2337/db07-0990]
  - 149 **Tan BK**, Adya R, Farhatullah S, Chen J, Lehnert H, Randeve HS. Metformin treatment may increase omentin-1 levels in women with polycystic ovary syndrome. *Diabetes* 2010; **59**: 3023-3031 [PMID: 20852028 DOI: 10.2337/db10-0124]
  - 150 **Duplus E**, Glorian M, Forest C. Fatty acid regulation of gene transcription. *J Biol Chem* 2000; **275**: 30749-30752 [PMID: 10934217 DOI: 10.1074/jbc.R000015200]
  - 151 **Food and Agriculture Organization of the United Nations**. Fats and fatty acids in human nutrition: Report of an expert consultation. Geneva, Rome: Food and Agriculture Organization of the United Nations, 2010
  - 152 **Gonzales AM**, Orlando RA. Role of adipocyte-derived lipoprotein lipase in adipocyte hypertrophy. *Nutr Metab (Lond)* 2007; **4**: 22 [PMID: 17971230 DOI: 10.1186/1743-7075-4-22]
  - 153 **Storch J**, Thumser AE. The fatty acid transport function of fatty acid-binding proteins. *Biochim Biophys Acta* 2000; **1486**: 28-44 [PMID: 10856711]
  - 154 **Hotamisligil GS**, Johnson RS, Distel RJ, Ellis R, Papaioannou VE, Spiegelman BM. Uncoupling of obesity from insulin resistance through a targeted mutation in aP2, the adipocyte fatty acid binding protein. *Science* 1996; **274**: 1377-1379 [PMID: 8910278]
  - 155 **Jakobsson A**, Westerberg R, Jacobsson A. Fatty acid elongases in mammals: their regulation and roles in metabolism. *Prog Lipid Res* 2006; **45**: 237-249 [PMID: 16564093 DOI: 10.1016/j.plipres.2006.01.004]
  - 156 **Wang Y**, Botolin D, Christian B, Busik J, Xu J, Jump DB. Tissue-specific, nutritional, and developmental regulation of rat fatty acid elongases. *J Lipid Res* 2005; **46**: 706-715 [PMID: 15654130 DOI: 10.1194/jlr.M400335-JLR200]
  - 157 **Kobayashi T**, Fujimori K. Very long-chain-fatty acids enhance adipogenesis through coregulation of Elovl3 and PPAR $\gamma$  in 3T3-L1 cells. *Am J Physiol Endocrinol Metab* 2012; **302**: E1461-E1471 [PMID: 22436697 DOI: 10.1152/ajpendo.00623.2011]
  - 158 **Blouet C**, Schwartz GJ. Hypothalamic nutrient sensing in the control of energy homeostasis. *Behav Brain Res* 2010; **209**: 1-12 [PMID: 20035790 DOI: 10.1016/j.bbr.2009.12.024]
  - 159 **Schmid B**, Rippmann JF, Tadayyon M, Hamilton BS. Inhibition of fatty acid synthase prevents preadipocyte differentiation. *Biochem Biophys Res Commun* 2005; **328**: 1073-1082 [PMID: 15707987 DOI: 10.1016/j.bbrc.2005.01.067]
  - 160 **Coleman RA**, Lee DP. Enzymes of triacylglycerol synthesis and their regulation. *Prog Lipid Res* 2004; **43**: 134-176 [PMID: 14654091]
  - 161 **Yu YH**, Zhang Y, Oelkers P, Sturley SL, Rader DJ, Ginsberg HN. Posttranscriptional control of the expression and function of diacylglycerol acyltransferase-1 in mouse adipocytes. *J Biol Chem* 2002; **277**: 50876-50884 [PMID: 12407108 DOI: 10.1074/jbc.M207353200]
  - 162 **Coleman R**, Bell RM. Triacylglycerol synthesis in isolated fat cells. Studies on the microsomal diacylglycerol acyltransferase activity using ethanol-dispersed diacylglycerols. *J Biol Chem* 1976; **251**: 4537-4543 [PMID: 947894]
  - 163 **Greenberg AS**, Egan JJ, Wek SA, Garty NB, Blanchette-Mackie EJ, Londos C. Perilipin, a major hormonally regulated adipocyte-specific phosphoprotein associated with the periphery of lipid storage droplets. *J Biol Chem* 1991; **266**: 11341-11346 [PMID: 2040638]
  - 164 **Rodriguez-Cuenca S**, Carobbio S, Velagapudi VR, Barbarroja N, Moreno-Navarrete JM, Tinahones FJ, Fernandez-Real JM, Orešic M, Vidal-Puig A. Peroxisome proliferator-activated receptor  $\gamma$ -dependent regulation of lipolytic nodes and metabolic flexibility. *Mol Cell Biol* 2012; **32**: 1555-1565 [PMID: 22310664 DOI: 10.1128/MCB.06154-11]
  - 165 **Unger RH**, Scherer PE. Gluttony, sloth and the metabolic syndrome: a roadmap to lipotoxicity. *Trends Endocrinol Metab* 2010; **21**: 345-352 [PMID: 20223680 DOI: 10.1016/j.tem.2010.01.009S1043-2760(10)00023-8]
  - 166 **Vidal-Puig A**, Unger RH. Special issue on lipotoxicity. *Biochim Biophys Acta* 2010; **1801**: 207-208 [PMID: 20045080 DOI: 10.1016/j.bbali.2009.12.010S1388-1981(09)00290-X]
  - 167 **Kolaczynski JW**, Nyce MR, Considine RV, Boden G, Nolan JJ, Henry R, Mudaliar SR, Olefsky J, Caro JF. Acute and chronic effects of insulin on leptin production in humans: Studies in vivo and in vitro. *Diabetes* 1996; **45**: 699-701 [PMID: 8621027]
  - 168 **Asterholm IW**, Scherer PE. Enhanced metabolic flexibility associated with elevated adiponectin levels. *Am J Pathol* 2010; **176**: 1364-1376 [PMID: 20093494 DOI: 10.2353/ajpath.2010.090647S0002-9440(10)60448-8]
  - 169 **Marchesini G**, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, McCullough AJ, Natale S, Forlani G, Melchionda N. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. *Diabetes* 2001; **50**: 1844-1850 [PMID: 11473047]
  - 170 **Xu H**, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* 2003; **112**: 1821-1830 [PMID: 14679177 DOI: 10.1172/JCI19451]
  - 171 **Sierra-Honigsmann MR**, Nath AK, Murakami C, Garcia-Cardeña G, Papapetropoulos A, Sessa WC, Madge LA, Schechner JS, Schwabb MB, Polverini PJ, Flores-Riveros JR. Biological action of leptin as an angiogenic factor. *Science* 1998; **281**: 1683-1686 [PMID: 9733517]
  - 172 **Maeda N**, Shimomura I, Kishida K, Nishizawa H, Matsuda M, Nagaretani H, Furuyama N, Kondo H, Takahashi M, Arita Y, Kumuro R, Ouchi N, Kihara S, Tochino Y, Okutomi K, Horie M, Takeda S, Aoyama T, Funahashi T, Matsuzawa Y. Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. *Nat Med* 2002; **8**: 731-737 [PMID: 12068289 DOI: 10.1038/nm724]
  - 173 **Wellen KE**, Hotamisligil GS. Obesity-induced inflammatory changes in adipose tissue. *J Clin Invest* 2003; **112**: 1785-1788 [PMID: 14679172 DOI: 10.1172/JCI20514]
  - 174 **van de Woestijne AP**, Monajemi H, Kalkhoven E, Visseren FL. Adipose tissue dysfunction and hypertriglyceridemia: mechanisms and management. *Obes Rev* 2011; **12**: 829-840 [PMID: 21749607 DOI: 10.1111/j.1467-789X.2011.00900.x]
  - 175 **Glass CK**, Witztum JL. Atherosclerosis. the road ahead. *Cell* 2001; **104**: 503-516 [PMID: 11239408]
  - 176 **Strissel KJ**, Stancheva Z, Miyoshi H, Perfield JW, DeFuria J, Jick Z, Greenberg AS, Obin MS. Adipocyte death, adipose tissue remodeling, and obesity complications. *Diabetes* 2007; **56**: 2910-2918 [PMID: 17848624 DOI: 10.2337/db07-0767]
  - 177 **Paniagua JA**, López-Miranda J, Pérez-Martínez P, Marín C, Vida JM, Fuentes F, Fernández de la Puebla RA, Pérez-Jiménez F. Oxidized-LDL levels are changed during short-term serum glucose variations and lowered with statin treatment in early Type



- 2 diabetes: a study of endothelial function and microalbuminuria. *Diabet Med* 2005; **22**: 1647-1656 [PMID: 16401307 DOI: 10.1111/j.1464-5491.2005.01703.x]
- 178 **Brown MS**, Goldstein JL. Receptor-mediated control of cholesterol metabolism. *Science* 1976; **191**: 150-154 [PMID: 174194]
- 179 **Steinberg D**. The cholesterol controversy is over. Why did it take so long? *Circulation* 1989; **80**: 1070-1078 [PMID: 2676235]
- 180 **Moreton JR**. Atherosclerosis and Alimentary Hyperlipemia. *Science* 1947; **106**: 190-191 [PMID: 17749166 DOI: 10.1126/science.106.2748.190]
- 181 **Hokanson JE**, Austin MA. Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. *J Cardiovasc Risk* 1996; **3**: 213-219 [PMID: 8836866]
- 182 **Patel A**, Barzi F, Jamrozik K, Lam TH, Ueshima H, Whitlock G, Woodward M; Asia Pacific Cohort Studies Collaboration. Serum triglycerides as a risk factor for cardiovascular diseases in the Asia-Pacific region. *Circulation* 2004; **110**: 2678-2686 [PMID: 15492305 DOI: 10.1161/01.CIR.0000145615.33955.83]
- 183 **Sarwar N**, Danesh J, Eiriksdottir G, Sigurdsson G, Wareham N, Bingham S, Boekholdt SM, Khaw KT, Gudnason V. Triglycerides and the risk of coronary heart disease: 10,158 incident cases among 262,525 participants in 29 Western prospective studies. *Circulation* 2007; **115**: 450-458 [PMID: 17190864 DOI: 10.1161/CIRCULATIONAHA.106.637793]
- 184 **Oram JF**, Lawn RM. ABCA1. The gatekeeper for eliminating excess tissue cholesterol. *J Lipid Res* 2001; **42**: 1173-1179 [PMID: 11483617]
- 185 **Chen W**, Chen G, Head DL, Mangelsdorf DJ, Russell DW. Enzymatic reduction of oxysterols impairs LXR signaling in cultured cells and the livers of mice. *Cell Metab* 2007; **5**: 73-79 [PMID: 17189208 DOI: 10.1016/j.cmet.2006.11.012]
- 186 **Calkin AC**, Tontonoz P. Liver x receptor signaling pathways and atherosclerosis. *Arterioscler Thromb Vasc Biol* 2010; **30**: 1513-1518 [PMID: 20631351 DOI: 10.1161/ATVBAHA.109.191197]
- 187 **Zhang Y**, Breevoort SR, Angdisen J, Fu M, Schmidt DR, Holmstrom SR, Kliewer SA, Mangelsdorf DJ, Schulman IG. Liver LXR $\alpha$  expression is crucial for whole body cholesterol homeostasis and reverse cholesterol transport in mice. *J Clin Invest* 2012; **122**: 1688-1699 [PMID: 22484817 DOI: 10.1172/JCI59817]
- 188 **Khera AV**, Cuchel M, de la Llera-Moya M, Rodrigues A, Burke MF, Jafri K, French BC, Phillips JA, Mucksavage ML, Wilensky RL, Mohler ER, Rothblat GH, Rader DJ. Cholesterol efflux capacity, high-density lipoprotein function, and atherosclerosis. *N Engl J Med* 2011; **364**: 127-135 [PMID: 21226578 DOI: 10.1056/NEJMoal001689]
- 189 **Oram JF**, Vaughan AM. ATP-Binding cassette cholesterol transporters and cardiovascular disease. *Circ Res* 2006; **99**: 1031-1043 [PMID: 17095732 DOI: 10.1161/01.RES.0000250171.54048.5c]
- 190 **Paniagua JA**, López-Miranda J, Jansen S, Zambrana JL, López Segura F, Jiménez Perepérez JA, Pérez-Jiménez F. Increased high-density lipoprotein-3 binding to leukocytes following weight loss and improved glycemic control in type 2 diabetic patients. *Metabolism* 2000; **49**: 692-697 [PMID: 10877191]
- 191 **Jeon JH**, Kim KY, Kim JH, Baek A, Cho H, Lee YH, Kim JW, Kim D, Han SH, Lim JS, Kim KI, Yoon DY, Kim SH, Oh GT, Kim E, Yang Y. A novel adipokine CTRP1 stimulates aldosterone production. *FASEB J* 2008; **22**: 1502-1511 [PMID: 18171693 DOI: 10.1096/fj.07-9412com]
- 192 **Engeli S**, Schling P, Gorzelnik K, Boschmann M, Janke J, Ailhaud G, Teboul M, Massiera F, Sharma AM. The adipose-tissue renin-angiotensin-aldosterone system: role in the metabolic syndrome? *Int J Biochem Cell Biol* 2003; **35**: 807-825 [PMID: 12676168 DOI: 10.1016/S1357-2725(02)00311-4]
- 193 **Reaven GM**. Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes* 1988; **37**: 1595-1607 [PMID: 3056758 DOI: 10.2337/diab.37.12.1595]
- 194 **Grundey SM**. Hypertriglyceridemia, insulin resistance, and the metabolic syndrome. *Am J Cardiol* 1999; **83**: 25F-29F [PMID: 10357572 DOI: 10.1016/S0002-9149(99)00211-8]
- 195 **Grundey SM**, Brewer HB, Cleeman JI, Smith SC, Lenfant C; American Heart Association; National Heart, Lung, and Blood Institute. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation* 2004; **109**: 433-438 [PMID: 14744958 DOI: 10.1161/01.CIR.000.0111245.75752.C6]
- 196 **National Heart, Lung, and Blood Institute**. Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults. The Evidence Report. Bethesda, Md: National Institutes of Health, 1968
- 197 **Williams RR**, Hopkins PN, Hunt SC, Schumacher MC, Elbein SC, Wilson DE, Stults BM, Wu LL, Hasstedt SJ, Lalouel JM. Familial dyslipidaemic hypertension and other multiple metabolic syndromes. *Ann Med* 1992; **24**: 469-475 [PMID: 1485941 DOI: 10.3109/07853899209166998]
- 198 **Stern MP**. Impaired glucose tolerance: Risk factor or diagnostic category. In: LeRoith D, Taylor SI, Olefsky JM, editors. *Diabetes Mellitus. A Fundamental and Clinical Text*. Philadelphia: Lippincott-Raven Publishers, 1996: 467-474
- 199 **Agatston AS**, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M, Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol* 1990; **15**: 827-832 [PMID: 2407762 DOI: 10.1016/0735-1097(90)90282-T]
- 200 **Alberti KG**, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 1998; **15**: 539-553 [PMID: 9686693 DOI: 10.1002/(SICI)1096-9136(199807)15: 7<539::AID-DIA668>3.0.CO;2-S]
- 201 **World Health Organization**. Definition, diagnosis and classification of diabetes mellitus and its complications: Report of a WHO Consultation. Part 1: Diagnosis and classification of diabetes mellitus. Geneva, Switzerland: World Health Organization, 1999. [accessed 2003 Dec 12]. Available from: URL: [http://whqlibdoc.who.int/hq/1999/WHO\\_NCD\\_NCS\\_99.2.pdf](http://whqlibdoc.who.int/hq/1999/WHO_NCD_NCS_99.2.pdf)
- 202 **Alberti KG**, Zimmet P, Shaw J. Metabolic syndrome—a new worldwide definition. A Consensus Statement from the International Diabetes Federation. *Diabet Med* 2006; **23**: 469-480 [PMID: 16681555 DOI: 10.1111/j.1464-5491.2006.01858.x]
- 203 **Tchernof A**, Lamarche B, Prud'Homme D, Nadeau A, Moorjani S, Labrie F, Lupien PJ, Després JP. The dense LDL phenotype. Association with plasma lipoprotein levels, visceral obesity, and hyperinsulinemia in men. *Diabetes Care* 1996; **19**: 629-637 [PMID: 8725863 DOI: 10.2337/diacare.19.6.629]
- 204 **Després JP**, Moorjani S, Lupien PJ, Tremblay A, Nadeau A, Bouchard C. Regional distribution of body fat, plasma lipoproteins, and cardiovascular disease. *Arteriosclerosis* 1990; **10**: 497-511 [PMID: 2196040 DOI: 10.1161/01.ATV.10.4.497]
- 205 **Kamel EG**, McNeill G, Han TS, Smith FW, Avenell A, Davidson L, Tothill P. Measurement of abdominal fat by magnetic resonance imaging, dual-energy X-ray absorptiometry and anthropometry in non-obese men and women. *Int J Obes Relat Metab Disord* 1999; **23**: 686-692 [PMID: 10454101 DOI: 10.1038/sj.ijo.0800904]
- 206 **Onat A**, Avci GS, Barlan MM, Uyarel H, Uzunlar B, Sansoy V. Measures of abdominal obesity assessed for visceral adiposity and relation to coronary risk. *Int J Obes Relat Metab Disord* 2004; **28**: 1018-1025 [PMID: 15197408 DOI: 10.1038/sj.ijo.0802695]
- 207 **Pouliot MC**, Després JP, Lemieux S, Moorjani S, Bouchard C, Tremblay A, Nadeau A, Lupien PJ. Waist circumference and abdominal sagittal diameter: best simple anthropometric indexes of abdominal visceral adipose tissue accumulation and related cardiovascular risk in men and women. *Am J Cardiol* 1994; **73**: 460-468 [PMID: 8141087 DOI: 10.1016/0002-9149(94)90676-9]
- 208 **Després JP**, Couillard C, Gagnon J, Bergeron J, Leon AS, Rao DC, Skinner JS, Wilmore JH, Bouchard C. Race, visceral adipose tissue, plasma lipids, and lipoprotein lipase activity in men and women: the

- Health, Risk Factors, Exercise Training, and Genetics (HERITAGE) family study. *Arterioscler Thromb Vasc Biol* 2000; **20**: 1932-1938 [PMID: 10938014 DOI: 10.1161/01.ATV.20.8.1932]
- 209 **Tan CE**, Ma S, Wai D, Chew SK, Tai ES. Can we apply the National Cholesterol Education Program Adult Treatment Panel definition of the metabolic syndrome to Asians? *Diabetes Care* 2004; **27**: 1182-1186 [PMID: 15111542 DOI: 10.2337/diacare.27.5.1182]
- 210 **Lear SA**, Toma M, Birmingham CL, Frohlich JJ. Modification of the relationship between simple anthropometric indices and risk factors by ethnic background. *Metabolism* 2003; **52**: 1295-1301 [PMID: 14564681 DOI: 10.1016/S0026-0495(03)00196-3]
- 211 **Carr MC**, Brunzell JD. Abdominal obesity and dyslipidemia in the metabolic syndrome: importance of type 2 diabetes and familial combined hyperlipidemia in coronary artery disease risk. *J Clin Endocrinol Metab* 2004; **89**: 2601-2607 [PMID: 15181030 DOI: 10.1210/jc.2004-0432]
- 212 **Lemieux I**, Pascot A, Prud'homme D, Alméras N, Bogaty P, Nadeau A, Bergeron J, Després JP. Elevated C-reactive protein: another component of the atherothrombotic profile of abdominal obesity. *Arterioscler Thromb Vasc Biol* 2001; **21**: 961-967 [PMID: 11397704]
- 213 **Yudkin JS**. Adipose tissue, insulin action and vascular disease: inflammatory signals. *Int J Obes Relat Metab Disord* 2003; **27** Suppl 3: S25-S28 [PMID: 14704740 DOI: 10.1038/sj.ijo.0802496]
- 214 **Devaraj S**, Rosenson RS, Jialal I. Metabolic syndrome: an appraisal of the pro-inflammatory and procoagulant status. *Endocrinol Metab Clin North Am* 2004; **33**: 431-453, table of contents [PMID: 15158528 DOI: 10.1016/j.ccl.2004.03.008]
- 215 **Matthews DR**, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; **28**: 412-419 [PMID: 3899825]
- 216 **Bergman RN**, Ider YZ, Bowden CR, Cobelli C. Quantitative estimation of insulin sensitivity. *Am J Physiol* 1979; **236**: E667-E677 [PMID: 443421]
- 217 **Nakajima M**, Sawada H, Yamada Y, Watanabe A, Tatsumi M, Yamashita J, Matsuda M, Sakaguchi T, Hirao T, Nakano H. The prognostic significance of amplification and overexpression of c-met and c-erb B-2 in human gastric carcinomas. *Cancer* 1999; **85**: 1894-1902 [PMID: 10223227 DOI: 10.1002/(SICI)1097-0142(19990501)85: 9<1894::AID-CNCR3>3.0.CO; 2-J]
- 218 **DeFronzo RA**, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 1979; **237**: E214-E223 [PMID: 382871]
- 219 **Tam CS**, Xie W, Johnson WD, Cefalu WT, Redman LM, Ravussin E. Defining insulin resistance from hyperinsulinemic-euglycemic clamps. *Diabetes Care* 2012; **35**: 1605-1610 [PMID: 22511259 DOI: 10.2337/dc11-2339dc11-2339]
- 220 **Ford ES**, Giles WH, Mokdad AH. Increasing prevalence of the metabolic syndrome among u.s. Adults. *Diabetes Care* 2004; **27**: 2444-2449 [PMID: 15451914 DOI: 10.2337/diacare.27.10.2444]
- 221 **Grundey SM**, Hansen B, Smith SC, Cleeman JI, Kahn RA; American Heart Association; National Heart, Lung, and Blood Institute; American Diabetes Association. Clinical management of metabolic syndrome: report of the American Heart Association/ National Heart, Lung, and Blood Institute/American Diabetes Association conference on scientific issues related to management. *Circulation* 2004; **109**: 551-556 [PMID: 14757684 DOI: 10.1161/01. CIR.0000112379.88385.67]
- 222 Report of the Dietary Guidelines Advisory Committee on the dietary guidelines for Americans, 2010: To the Secretary of Agriculture and the Secretary of Health and Human Services. Washington, D.C.: United States Department of Agriculture, United States Department of Health and Human Services, 2010
- 223 **Fogli-Cawley JJ**, Dwyer JT, Saltzman E, McCullough ML, Troy LM, Meigs JB, Jacques PF. The 2005 Dietary Guidelines for Americans and risk of the metabolic syndrome. *Am J Clin Nutr* 2007; **86**: 1193-1201 [PMID: 17921402]
- 224 **Fogli-Cawley JJ**, Dwyer JT, Saltzman E, McCullough ML, Troy LM, Meigs JB, Jacques PF. The 2005 Dietary Guidelines for Americans and insulin resistance in the Framingham Offspring Cohort. *Diabetes Care* 2007; **30**: 817-822 [PMID: 17259479 DOI: 10.2337/dc06-1927]
- 225 **Office of Disease Prevention and Health Promotion**. Scientific report of the 2015 dietary guidelines advisory committee. [accessed 2015 Feb 20]. Available from: URL: <http://www.health.gov/dietaryguidelines/2015-scientific-report/PDFs/Scientific-Report-of-the-2015-Dietary-Guidelines-Advisory-Committee.pdf>
- 226 **Esposito K**, Marfella R, Ciotola M, Di Palo C, Giugliano F, Giugliano G, D'Armiento M, D'Andrea F, Giugliano D. Effect of a mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. *JAMA* 2004; **292**: 1440-1446 [PMID: 15383514 DOI: 10.1001/jama.292.12.1440]
- 227 **Babio N**, Toledo E, Estruch R, Ros E, Martínez-González MA, Castañer O, Bulló M, Corella D, Arós F, Gómez-Gracia E, Ruiz-Gutiérrez V, Fiol M, Lapetra J, Lamuela-Raventós RM, Serra-Majem L, Pintó X, Basora J, Sorlí JV, Salas-Salvadó J. Mediterranean diets and metabolic syndrome status in the PREDIMED randomized trial. *CMAJ* 2014; **186**: E649-E657 [PMID: 25316904 DOI: 10.1503/cmaj.140764]
- 228 **Storlien LH**, Baur LA, Kriketos AD, Pan DA, Cooney GJ, Jenkins AB, Calvert GD, Campbell LV. Dietary fats and insulin action. *Diabetologia* 1996; **39**: 621-631 [PMID: 8781757]
- 229 **Feskens EJ**, Virtanen SM, Räsänen L, Tuomilehto J, Stengård J, Pekkanen J, Nissinen A, Kromhout D. Dietary factors determining diabetes and impaired glucose tolerance. A 20-year follow-up of the Finnish and Dutch cohorts of the Seven Countries Study. *Diabetes Care* 1995; **18**: 1104-1112 [PMID: 7587845]
- 230 **Melanson EL**, Astrup A, Donahoo WT. The relationship between dietary fat and fatty acid intake and body weight, diabetes, and the metabolic syndrome. *Ann Nutr Metab* 2009; **55**: 229-243 [PMID: 19752544 DOI: 10.1159/000229004]
- 231 **Orchard TJ**, Temprosa M, Goldberg R, Haffner S, Ratner R, Marcovina S, Fowler S; Diabetes Prevention Program Research Group. The effect of metformin and intensive lifestyle intervention on the metabolic syndrome: the Diabetes Prevention Program randomized trial. *Ann Intern Med* 2005; **142**: 611-619 [PMID: 15838067]
- 232 **Tuomilehto J**, Lindström J, Eriksson JG, Valle TT, Hämäläinen H, Ilanne-Parikka P, Keinänen-Kiukaanniemi S, Laakso M, Louheranta A, Rastas M, Salminen V, Uusitupa M. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N Engl J Med* 2001; **344**: 1343-1350 [PMID: 11333990 DOI: 10.1056/NEJM200105033441801]
- 233 **Praagman J**, Beulens JW, Alsema M, Zock PL, Wanders AJ, Sluijs I, van der Schouw YT. The association between dietary saturated fatty acids and ischemic heart disease depends on the type and source of fatty acid in the European Prospective Investigation into Cancer and Nutrition-Netherlands cohort. *Am J Clin Nutr* 2016; **103**: 356-365 [PMID: 26791181 DOI: 10.3945/ajcn.115.122671]
- 234 **Samaha FF**, Iqbal N, Seshadri P, Chicano KL, Daily DA, McGrory J, Williams T, Williams M, Gracely EJ, Stern L. A low-carbohydrate as compared with a low-fat diet in severe obesity. *N Engl J Med* 2003; **348**: 2074-2081 [PMID: 12761364 DOI: 10.1056/NEJMoa022637]
- 235 **Gardner CD**, Kiazand A, Alhassan S, Kim S, Stafford RS, Balise RR, Kraemer HC, King AC. Comparison of the Atkins, Zone, Ornish, and LEARN diets for change in weight and related risk factors among overweight premenopausal women: the A TO Z Weight Loss Study: a randomized trial. *JAMA* 2007; **297**: 969-977 [PMID: 17341711 DOI: 10.1001/jama.297.9.969]
- 236 **Larsen TM**, Dalskov SM, van Baak M, Jebb SA, Papadaki A, Pfeiffer AF, Martinez JA, Handjieva-Darlenska T, Kunešová M, Pihlsgård M, Stender S, Holst C, Saris WH, Astrup A. Diets with high or low protein content and glycemic index for weight-loss maintenance. *N Engl J Med* 2010; **363**: 2102-2113 [PMID: 21105792]

DOI: 10.1056/NEJMoa1007137]

- 237 **Paniagua JA**, de la Sacristana AG, Sánchez E, Romero I, Vidal-Puig A, Berral FJ, Escribano A, Moyano MJ, Pérez-Martínez P, López-Miranda J, Pérez-Jiménez F. A MUFA-rich diet improves postprandial glucose, lipid and GLP-1 responses in insulin-resistant subjects. *J Am Coll Nutr* 2007; **26**: 434-444 [PMID: 17914131]
- 238 **Du H**, van der A DL, Boshuizen HC, Forouhi NG, Wareham NJ, Halkjaer J, Tjønneland A, Overvad K, Jakobsen MU, Boeing H, Buijsse B, Masala G, Palli D, Sørensen TI, Saris WH, Feskens EJ. Dietary fiber and subsequent changes in body weight and waist circumference in European men and women. *Am J Clin Nutr* 2010; **91**: 329-336 [PMID: 20016015 DOI: 10.3945/ajcn.2009.28191]
- 239 **Romaguera D**, Angquist L, Du H, Jakobsen MU, Forouhi NG, Halkjaer J, Feskens EJ, van der A DL, Masala G, Steffen A, Palli D, Wareham NJ, Overvad K, Tjønneland A, Boeing H, Riboli E, Sørensen TI. Dietary determinants of changes in waist circumference adjusted for body mass index - a proxy measure of visceral adiposity. *PLoS One* 2010; **5**: e11588 [PMID: 20644647 DOI: 10.1371/journal.pone.0011588]
- 240 **Parnell JA**, Reimer RA. Weight loss during oligofructose supplementation is associated with decreased ghrelin and increased peptide YY in overweight and obese adults. *Am J Clin Nutr* 2009; **89**: 1751-1759 [PMID: 19386741 DOI: 10.3945/ajcn.2009.27465]
- 241 **Cani PD**, Joly E, Horsmans Y, Delzenne NM. Oligofructose promotes satiety in healthy human: a pilot study. *Eur J Clin Nutr* 2006; **60**: 567-572 [PMID: 16340949 DOI: 10.1038/sj.ejcn.1602350]
- 242 **Field AE**, Willett WC, Lissner L, Colditz GA. Dietary fat and weight gain among women in the Nurses' Health Study. *Obesity* (Silver Spring) 2007; **15**: 967-976 [PMID: 17426332 DOI: 10.1038/oby.2007.616]
- 243 **Soriguer F**, Almaraz MC, García-Almeida JM, Cardona I, Linares F, Morcillo S, García-Escobar E, Dobarganes MC, Oliveira G, Hernando V, Valdes S, Ruiz-de-Adana MS, Esteve I, Rojo-Martínez G. Intake and home use of olive oil or mixed oils in relation to healthy lifestyles in a Mediterranean population. Findings from the prospective Pizarra study. *Br J Nutr* 2010; **103**: 114-122 [PMID: 19747416 DOI: 10.1017/S0007114509991498]
- 244 **Piers LS**, Walker KZ, Stoney RM, Soares MJ, O'Dea K. Substitution of saturated with monounsaturated fat in a 4-week diet affects body weight and composition of overweight and obese men. *Br J Nutr* 2003; **90**: 717-727 [PMID: 13129479]
- 245 **Micallef M**, Munro I, Phang M, Garg M. Plasma n-3 Polyunsaturated Fatty Acids are negatively associated with obesity. *Br J Nutr* 2009; **102**: 1370-1374 [PMID: 19454127 DOI: 10.1017/S0007114509382173]
- 246 **Garaulet M**, Pérez-Llamas F, Pérez-Ayala M, Martínez P, de Medina FS, Tebar FJ, Zamora S. Site-specific differences in the fatty acid composition of abdominal adipose tissue in an obese population from a Mediterranean area: relation with dietary fatty acids, plasma lipid profile, serum insulin, and central obesity. *Am J Clin Nutr* 2001; **74**: 585-591 [PMID: 11684525]
- 247 **Camargo A**, Meneses ME, Perez-Martinez P, Delgado-Lista J, Jimenez-Gomez Y, Cruz-Teno C, Tinahones FJ, Paniagua JA, Perez-Jimenez F, Roche HM, Malagon MM, Lopez-Miranda J. Dietary fat differentially influences the lipids storage on the adipose tissue in metabolic syndrome patients. *Eur J Nutr* 2014; **53**: 617-626 [PMID: 23922010 DOI: 10.1007/s00394-013-0570-2]
- 248 **Gaullier JM**, Halse J, Høye K, Kristiansen K, Fagertun H, Vik H, Gudmundsen O. Conjugated linoleic acid supplementation for 1 y reduces body fat mass in healthy overweight humans. *Am J Clin Nutr* 2004; **79**: 1118-1125 [PMID: 15159244]
- 249 **Gaullier JM**, Halse J, Høivik HO, Høye K, Syvertsen C, Nurminiemi M, Hassfeld C, Einerhand A, O'Shea M, Gudmundsen O. Six months supplementation with conjugated linoleic acid induces regional-specific fat mass decreases in overweight and obese. *Br J Nutr* 2007; **97**: 550-560 [PMID: 17313718 DOI: 10.1017/S0007114507381324]
- 250 **Smedman A**, Vessby B. Conjugated linoleic acid supplementation in humans--metabolic effects. *Lipids* 2001; **36**: 773-781 [PMID: 11592727 DOI: 10.1007/s11745-001-0784-7]
- 251 **Vessby B**, Uusitupa M, Hermansen K, Riccardi G, Rivelles AA, Tapsell LC, Näslén C, Berglund L, Louheranta A, Rasmussen BM, Calvert GD, Maffetone A, Pedersen E, Gustafsson IB, Storlien LH. Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: The KANWU Study. *Diabetologia* 2001; **44**: 312-319 [PMID: 11317662 DOI: 10.1007/s001250051620]
- 252 **Salas J**, López-Miranda J, Jansen S, Zambrana JL, Castro P, Paniagua JA, Blanco A, López Segura F, Jiménez Perepérez JA, Pérez Jiménez F. [The diet rich in monounsaturated fat modifies in a beneficial way carbohydrate metabolism and arterial pressure]. *Med Clin (Barc)* 1999; **113**: 765-769 [PMID: 10680139]
- 253 **Pérez-Jiménez F**, López-Miranda J, Pinillos MD, Gómez P, Paz-Rojas E, Montilla P, Marín C, Velasco MJ, Blanco-Molina A, Jiménez Perepérez JA, Ordovás JM. A Mediterranean and a high-carbohydrate diet improve glucose metabolism in healthy young persons. *Diabetologia* 2001; **44**: 2038-2043 [PMID: 11719836 DOI: 10.1007/s001250100009]
- 254 **Tierney AC**, McMonagle J, Shaw DI, Gulseth HL, Helal O, Saris WH, Paniagua JA, Gołabek-Leszczynska I, Defoort C, Williams CM, Karström B, Vessby B, Dembinska-Kiec A, López-Miranda J, Blaak EE, Drevon CA, Gibney MJ, Lovegrove JA, Roche HM. Effects of dietary fat modification on insulin sensitivity and on other risk factors of the metabolic syndrome--LIPGENE: a European randomized dietary intervention study. *Int J Obes (Lond)* 2011; **35**: 800-809 [PMID: 20938439 DOI: 10.1038/ijo.2010.209]
- 255 **Yubero-Serrano EM**, Delgado-Lista J, Tierney AC, Perez-Martinez P, Garcia-Rios A, Alcala-Diaz JF, Castaño JP, Tinahones FJ, Drevon CA, Defoort C, Blaak EE, Dembinska-Kiec A, Risérus U, Lovegrove JA, Perez-Jimenez F, Roche HM, Lopez-Miranda J. Insulin resistance determines a differential response to changes in dietary fat modification on metabolic syndrome risk factors: the LIPGENE study. *Am J Clin Nutr* 2015; **102**: 1509-1517 [PMID: 26561628 DOI: 10.3945/ajcn.115.111286]
- 256 **Atkinson FS**, Foster-Powell K, Brand-Miller JC. International tables of glycemic index and glycemic load values: 2008. *Diabetes Care* 2008; **31**: 2281-2283 [PMID: 18835944 DOI: 10.2337/dc08-1239]
- 257 **Uyeda K**, Repa JJ. Carbohydrate response element binding protein, ChREBP, a transcription factor coupling hepatic glucose utilization and lipid synthesis. *Cell Metab* 2006; **4**: 107-110 [PMID: 16890538 DOI: 10.1016/j.cmet.2006.06.008]
- 258 **McMillan-Price J**, Petocz P, Atkinson F, O'Neill K, Samman S, Steinbeck K, Caterson I, Brand-Miller J. Comparison of 4 diets of varying glycemic load on weight loss and cardiovascular risk reduction in overweight and obese young adults: a randomized controlled trial. *Arch Intern Med* 2006; **166**: 1466-1475 [PMID: 16864756 DOI: 10.1001/archinte.166.14.1466]
- 259 **Jeppesen J**, Hollenbeck CB, Zhou MY, Coulston AM, Jones C, Chen YD, Reaven GM. Relation between insulin resistance, hyperinsulinemia, postheparin plasma lipoprotein lipase activity, and postprandial lipemia. *Arterioscler Thromb Vasc Biol* 1995; **15**: 320-324 [PMID: 7749841 DOI: 10.1161/01.ATV.15.3.320]
- 260 **Panarotto D**, Rémillard P, Bouffard L, Maheux P. Insulin resistance affects the regulation of lipoprotein lipase in the postprandial period and in an adipose tissue-specific manner. *Eur J Clin Invest* 2002; **32**: 84-92 [PMID: 11895454 DOI: 10.1046/j.1365-2362.2002.00945.x]
- 261 **Paniagua JA**, Pérez-Martínez P, Gjelstad IM, Tierney AC, Delgado-Lista J, Defoort C, Blaak EE, Risérus U, Drevon CA, Kieć-Wilk B, Lovegrove JA, Roche HM, López-Miranda J. A low-fat high-carbohydrate diet supplemented with long-chain n-3 PUFA reduces the risk of the metabolic syndrome. *Atherosclerosis* 2011; **218**: 443-450 [PMID: 21839455 DOI: 10.1016/j.atherosclerosis.2011.07.03]
- 262 **Ueshima H**, Stamler J, Elliott P, Chan Q, Brown IJ, Carnethon MR, Daviglus ML, He K, Moag-Stahlberg A, Rodriguez BL, Steffen LM, Van Horn L, Yarnell J, Zhou B. Food omega-3 fatty acid intake of individuals (total, linolenic acid, long-chain) and their blood



- pressure: INTERMAP study. *Hypertension* 2007; **50**: 313-319 [PMID: 17548718 DOI: 10.1161/HYPERTENSIONAHA.107.090720]
- 263 **Deckelbaum RJ**, Worgall TS, Seo T. n-3 fatty acids and gene expression. *Am J Clin Nutr* 2006; **83**: 1520S-1525S [PMID: 16841862]
- 264 **Chandalia M**, Garg A, Lutjohann D, von Bergmann K, Grundy SM, Brinkley LJ. Beneficial effects of high dietary fiber intake in patients with type 2 diabetes mellitus. *N Engl J Med* 2000; **342**: 1392-1398 [PMID: 10805824 DOI: 10.1056/NEJM200005113421903]
- 265 **Berg AH**, Combs TP, Du X, Brownlee M, Scherer PE. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nat Med* 2001; **7**: 947-953 [PMID: 11479628 DOI: 10.1038/90992]
- 266 **Silva FM**, de Almeida JC, Feoli AM. Effect of diet on adiponectin levels in blood. *Nutr Rev* 2011; **69**: 599-612 [PMID: 21967160 DOI: 10.1111/j.1753-4887.2011.00414.x]
- 267 **Reseland JE**, Anderssen SA, Solvoll K, Hjermann I, Urdal P, Holme I, Drevon CA. Effect of long-term changes in diet and exercise on plasma leptin concentrations. *Am J Clin Nutr* 2001; **73**: 240-245 [PMID: 11157319]
- 268 **Karbowska J**, Kochan Z. Intermittent fasting up-regulates Fsp27/Cidec gene expression in white adipose tissue. *Nutrition* 2012; **28**: 294-299 [PMID: 21996045 DOI: 10.1016/j.nut.2011.06.009]
- 269 **Belfort R**, Harrison SA, Brown K, Darland C, Finch J, Hardies J, Balas B, Gastaldelli A, Tio F, Pulcini J, Berria R, Ma JZ, Dwivedi S, Havranek R, Fincke C, DeFronzo R, Bannayan GA, Schenker S, Cusi K. A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. *N Engl J Med* 2006; **355**: 2297-2307 [PMID: 17135584 DOI: 10.1056/NEJMoa060326]
- 270 **Camargo A**, Meneses ME, Rangel-Zuñiga OA, Perez-Martinez P, Marin C, Delgado-Lista J, Paniagua JA, Tinahones FJ, Roche H, Malagon MM, Perez-Jimenez F, Lopez-Miranda J. Endoplasmic reticulum stress in adipose tissue determines postprandial lipoprotein metabolism in metabolic syndrome patients. *Mol Nutr Food Res* 2013; **57**: 2166-2176 [PMID: 23934773 DOI: 10.1002/mnfr.201300036]
- 271 **Peña-Orihuela P**, Camargo A, Rangel-Zuñiga OA, Perez-Martinez P, Cruz-Teno C, Delgado-Lista J, Yubero-Serrano EM, Paniagua JA, Tinahones FJ, Malagon MM, Roche HM, Perez-Jimenez F, Lopez-Miranda J. Antioxidant system response is modified by dietary fat in adipose tissue of metabolic syndrome patients. *J Nutr Biochem* 2013; **24**: 1717-1723 [PMID: 23647888 DOI: 10.1016/j.jnutbio.2013.02.012]
- 272 **Meneses ME**, Camargo A, Perez-Martinez P, Delgado-Lista J, Cruz-Teno C, Jimenez-Gomez Y, Paniagua JA, Gutierrez-Mariscal FM, Tinahones FJ, Vidal-Puig A, Roche HM, Perez-Jimenez F, Malagon MM, Lopez-Miranda J. Postprandial inflammatory response in adipose tissue of patients with metabolic syndrome after the intake of different dietary models. *Mol Nutr Food Res* 2011; **55**: 1759-1770 [PMID: 22144044 DOI: 10.1002/mnfr.201100200]
- 273 **López-Miranda J**, Pérez-Jiménez F, Ros E, De Caterina R, Badimón L, Covas MI, Escribá E, Ordovás JM, Soriguer F, Abiá R, de la Lastra CA, Battino M, Corella D, Chamorro-Quirós J, Delgado-Lista J, Giugliano D, Esposito K, Estruch R, Fernandez-Real JM, Gaforio JJ, La Vecchia C, Lairon D, López-Segura F, Mata P, Menéndez JA, Muriana FJ, Osada J, Panagiotakos DB, Paniagua JA, Pérez-Martinez P, Perona J, Peinado MA, Pineda-Priego M, Poulsen HE, Quiles JL, Ramirez-Tortosa MC, Ruano J, Serra-Majem L, Solá R, Solanas M, Solfrizzi V, de la Torre-Fornell R, Trichopoulou A, Uceda M, Villalba-Montoro JM, Villar-Ortiz JR, Visioli F, Yiannakouris N. Olive oil and health: summary of the II international conference on olive oil and health consensus report, Jaén and Córdoba (Spain) 2008. *Nutr Metab Cardiovasc Dis* 2010; **20**: 284-294 [PMID: 20303720 DOI: 10.1016/j.numecd.2009.12.007]
- 274 **Freiberg MS**, Cabral HJ, Heeren TC, Vasan RS, Curtis Ellison R; Third National Health and Nutrition Examination Survey. Alcohol consumption and the prevalence of the Metabolic Syndrome in the US.: a cross-sectional analysis of data from the Third National Health and Nutrition Examination Survey. *Diabetes Care* 2004; **27**: 2954-2959 [PMID: 15562213]
- 275 **Weisberg SP**, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 2003; **112**: 1796-1808 [PMID: 14679176 DOI: 10.1172/JCI19246]
- 276 **Bulló M**, García-Lorda P, Megias I, Salas-Salvadó J. Systemic inflammation, adipose tissue tumor necrosis factor, and leptin expression. *Obes Res* 2003; **11**: 525-531 [PMID: 12690081 DOI: 10.1038/oby.2003.74]
- 277 **Prieur X**, Mok CY, Velagapudi VR, Núñez V, Fuentes L, Montaner D, Ishikawa K, Camacho A, Barbarroja N, O'Rahilly S, Sethi JK, Dopazo J, Orešič M, Ricote M, Vidal-Puig A. Differential lipid partitioning between adipocytes and tissue macrophages modulates macrophage lipotoxicity and M2/M1 polarization in obese mice. *Diabetes* 2011; **60**: 797-809 [PMID: 21266330 DOI: 10.2337/db10-0705]
- 278 **Hotamisligil GS**, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor- $\alpha$ : direct role in obesity-linked insulin resistance. *Science* 1993; **259**: 87-91 [PMID: 7678183 DOI: 10.1126/science.7678183]
- 279 **Rosen BS**, Cook KS, Yaglom J, Groves DL, Volanakis JE, Damm D, White T, Spiegelman BM. Adipsin and complement factor D activity: an immune-related defect in obesity. *Science* 1989; **244**: 1483-1487 [PMID: 2734615 DOI: 10.1126/science.2734615]
- 280 **Kasbi Chadli F**, Andre A, Prieur X, Loirand G, Meynier A, Krempf M, Nguyen P, Ouguerram K. n-3 PUFA prevent metabolic disturbances associated with obesity and improve endothelial function in golden Syrian hamsters fed with a high-fat diet. *Br J Nutr* 2012; **107**: 1305-1315 [PMID: 21920060 DOI: 10.1017/S0007114511004387]
- 281 **Guilherme A**, Virbasius JV, Puri V, Czech MP. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. *Nat Rev Mol Cell Biol* 2008; **9**: 367-377 [PMID: 18401346 DOI: 10.1038/nrm2391]
- 282 **Sun K**, Kusminski CM, Scherer PE. Adipose tissue remodeling and obesity. *J Clin Invest* 2011; **121**: 2094-2101 [PMID: 21633177 DOI: 10.1172/JCI45887]
- 283 **Hotamisligil GS**, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor- $\alpha$  in human obesity and insulin resistance. *J Clin Invest* 1995; **95**: 2409-2415 [PMID: 7738205 DOI: 10.1172/JCI117936]
- 284 **Donnelly KL**, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *J Clin Invest* 2005; **115**: 1343-1351 [PMID: 15864352 DOI: 10.1172/JCI23621]
- 285 **Hotamisligil GS**. Inflammation, TNF- $\alpha$  and insulin resistance. Philadelphia, PA, United States: Lippincott-Raven Publishers, 2003
- 286 **Hirosumi J**, Tuncman G, Chang L, Görgün CZ, Uysal KT, Maeda K, Karin M, Hotamisligil GS. A central role for JNK in obesity and insulin resistance. *Nature* 2002; **420**: 333-336 [PMID: 12447443 DOI: 10.1038/nature01137]
- 287 **Després JP**, Lemieux I, Bergeron J, Pibarot P, Mathieu P, Larose E, Rodés-Cabau J, Bertrand OF, Poirier P. Abdominal obesity and the metabolic syndrome: contribution to global cardiometabolic risk. *Arterioscler Thromb Vasc Biol* 2008; **28**: 1039-1049 [PMID: 18356555 DOI: 10.1161/ATVBAHA.107.159228]
- 288 **Kim JY**, van de Wall E, Laplante M, Azzara A, Trujillo ME, Hofmann SM, Schraw T, Durand JL, Li H, Li G, Jelicks LA, Mehler MF, Hui DY, Deshaies Y, Shulman GI, Schwartz GJ, Scherer PE. Obesity-associated improvements in metabolic profile through expansion of adipose tissue. *J Clin Invest* 2007; **117**: 2621-2637 [PMID: 17717599 DOI: 10.1172/JCI31021]
- 289 **Adiels M**, Borén J, Caslake MJ, Stewart P, Soro A, Westerbacka J, Wennberg B, Olofsson SO, Packard C, Taskinen MR. Overproduction of VLDL1 driven by hyperglycemia is a dominant feature of diabetic dyslipidemia. *Arterioscler Thromb Vasc Biol* 2005; **25**: 1697-1703 [PMID: 15947244 DOI: 10.1161/01.ATV.0000172689.53992.25]
- 290 **Rohatgi A**, Khera A, Berry JD, Givens EG, Ayers CR, Wedin KE, Neeland IJ, Yuhanna IS, Rader DR, de Lemos JA, Shaul PW. HDL cholesterol efflux capacity and incident cardiovascular events. *N*



*Engl J Med* 2014; **371**: 2383-2393 [PMID: 25404125 DOI: 10.1056/NEJMoa1409065]

291 **Kontush A**, Chapman MJ. Functionally defective high-density

lipoprotein: a new therapeutic target at the crossroads of dyslipidemia, inflammation, and atherosclerosis. *Pharmacol Rev* 2006; **58**: 342-374 [PMID: 16968945 DOI: 10.1124/pr.58.3.1]

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## Sleep, circadian dysrhythmia, obesity and diabetes

Gumpeny Ramachandra Sridhar, Narasimhadevara Santhi Nirmala Sanjana

Gumpeny Ramachandra Sridhar, Endocrine and Diabetes Centre, Visakhapatnam 530002, India

Narasimhadevara Santhi Nirmala Sanjana, MGM Medical College, Navi Mumbai 410209, India

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**Correspondence to:** Gumpeny Ramachandra Sridhar, MD, DM, FACE, FRCP, Endocrine and Diabetes Centre, 15-12-15 Krishnanagar, Visakhapatnam 530002, India. [sridharvizag@gmail.com](mailto:sridharvizag@gmail.com)  
Telephone: +91-891-2566301  
Fax: +91-891-2509427

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### Abstract

Synchrony of biological processes with environmental cues developed over millennia to match growth, reproduction and senescence. This entails a complex interplay of genetic, metabolic, chemical, light, hormonal and

hedonistic factors across life forms. Sleep is one of the most prominent rhythms where such a match is established. Over the past 100 years or so, it has been possible to disturb the synchrony between sleep-wake cycle and environmental cues. Development of electric lights, shift work and continual accessibility of the internet has disrupted this match. As a result, many non-communicable diseases such as obesity, insulin resistance, type 2 diabetes, coronary artery disease and malignancies have been attributed in part to such disruption. In this presentation a review is made of the origin and evolution of sleep studies, the pathogenic mediators for such asynchrony, clinical evidence and relevance and suggested management options to deal with the disturbances.

**Key words:** Insulin resistance; Chronotype; Obesity; Evolution; Clock; Shift work

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**Core tip:** Humans evolved to match external environment with internal metabolism. Day-night cycle is an important rhythm to achieve synchrony. A central clock interacts with peripheral clocks in various parts of the body. Reduced sleep, shift work and inappropriate exposure to light during sleep hours disturb this rhythm leading to abnormalities such as obesity, insulin resistance and type 2 diabetes. Understanding the complex interactions of the various factors involved in this system can help in the prevention and in treatment of such adverse effects.

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### BACKGROUND

Sleep is the most pronounced human rhythmic activity in humans. Rhythmicity of biological systems developed

over the course of evolution so that adaptation occurred to changes of environment with the physiology of organisms<sup>[1,2]</sup>. Such alignment ensured their survival, and is a powerful evolutionary pressure. While it was recognized that altered core circadian clock genes alters sleep architecture and duration, targeted deletion of *BMAL1/Mop3* gene, which is a partner to *CLOCK* resulted in disturbances in generation of sleep and wakefulness. These were in addition to wakefulness and the timing of vigilance<sup>[3]</sup>. Besides, the *CLOCK* transcription factor is a key component of the circadian clock in the hypothalamic suprachiasmatic nucleus, that leads to attenuation in feeding rhythm leading to hyperphagia, obesity and metabolic syndrome in mice having mutant homozygous *CLOCK* genes<sup>[4]</sup>. The interaction between genes of the circadian clock and of metabolic genes is mediated by the remodeling of histone proteins<sup>[5]</sup>.

Despite human beings now having the ability to alter the light-dark cycle, the strong role of circadian clock is still evident on the social and metabolic effects. From the first human experimental work of Jürgen Aschoff emerging studies suggest the role of lunar cycles could also be involved, operating through changes in physical activity<sup>[6,7]</sup>.

Such asynchrony of social and biological clocks leads to obesity, diabetes, cardiovascular disease and cancer<sup>[6]</sup>. Disturbed daily rhythms reflect in expression of different gene groups as well, suggesting a close relation between rhythmicity and biological well-being<sup>[8]</sup>.

The relation of *CLOCK* transcription factor and various metabolic abnormalities has been reported in the past few years. Gene variants of the *CLOCK* transcription factor was shown to be associated with nonalcoholic fatty liver disease (NAFLD), a condition linked to insulin resistance<sup>[9]</sup>. Among 136 subjects with NAFLD and 64 controls, rs11932595 and rs6843722 showed a significant association with NAFLD. This suggests a potential relation between *CLOCK* polymorphisms and NAFLD. A more recent study showed that variants of the *CLOCK* gene could have a role in the expression of obesity and other metabolic traits. Unrelated subjects who were lean (*n*: 715) and obese (*n*: 391) were recruited from a cross sectional population based cohort. SNPs with minor allele frequency were genotyped. Four tag SNP genotype frequencies (rs1554483, rs6843722, rs6850524 and rs4864548) showed associations with overweight or obesity<sup>[10]</sup>. The fine-tuning of the body's clock evolved to conserve energy and to improve efficiency. Such synchronization allows one to anticipate and respond to environmental alterations<sup>[11]</sup>.

Obesity and type 2 diabetes have become leading causes of disease and death world-wide. Part of the reason for the epidemic appears to be desynchrony over the last 100 years between the body's endogenous clock located in the anterior hypothalamic suprachiasmatic nuclei, which responds to the dark-light cycle and the iatrogenic disturbance of such rhythmicity. The central clock is aided by similar clocks in the periphery at the liver, fat tissue and gastrointestinal tract, which together,

regulate energy metabolism *via* enzymatic activation or suppression<sup>[12]</sup>. The integration of clock mechanism with metabolism occurs through hormones, nutrients and meal timings.

Recent evidence has shown that variation in genes related to circadian rhythm is associated with extreme obesity, which can be modified by variants in *CLOCK* genes. Mutations of genes in hypothalamus, a key regulator of energy intake, result in early life obesity. To identify gene variants in the background of obesity, a selected phenotype with extreme obesity was taken. One hundred and sixty-six genes functionally related to the hypothalamus, were subjected to complete exome sequencing in 30 extremely obese subjects, for novel rare indel, nonsense and missense variants. The authors identified six novel rare deleterious missense variants (in genes for *BAIAP3*, *NBEA*, *PRRC2A*, *RYR1*, *SIM1* and *TRH*; a novel indel variant was found in *LEPR*). Both rare and common variants of genes thus regulate circadian food intake and hypothalamic signal process are involved in extreme obesity<sup>[13]</sup>.

Similarly there was an association of habitual sleep duration, BMI, nutrient intake and *CLOCK* variants. In an "inverse-variance weighted, fixed-effect meta-analysis of adjusted associations of sleep duration and BMI and macronutrient intake as percentages of total energy" interactions were studied with *CLOCK* variants<sup>[14]</sup>. Data were obtained from nine cohort subjects (*n*: 14896). Interestingly there was a significant association of lower intake of saturated fatty acids and sleep duration among younger adults, and with a lower intake of carbohydrates, higher total fats, higher PUFA intake in older women. In addition interactions were seen between sleep duration and rs12649507 on PUFA intake and with sleep duration and rs6858749 on protein intake. The results imply suggest that longer duration of sleep can attenuate genetic predisposition to obesity acting through intake of appropriate diet<sup>[14]</sup>.

Along the same lines, associations of circadian clock and SIRTUIN1 (*SIRT1*) dependent functions may lead to evening preference of food intake and resistance to weight loss. *SIRT1* (rs1467568) and *CLOCK* (3111T > C, rs1801260) were genotyped in a large cohort of subjects who were overweight or obese (*n*: 1465). On follow up for weight loss *via* behavior therapy, those with minor alleles of *SIRT1* and *CLOCK* loci had higher resistance to weight loss compared to homozygotes. Subjects carrying the R genotype had elevated levels of plasma ghrelin, which could modulate the gene variants in the resistance to weight loss<sup>[15]</sup>.

In addition to their putative role in sleep timing, depression and obesity, variant *CLOCK* genes could also influence the duration of sleep. From a sample of 77000 subjects administered Munich ChronoType questionnaire, a subsample on follow up was evaluated by a two-stage design, linkage disequilibrium based association study with short sleep (< 7 h) and long (> 8.5 h) sleep. In the discovery sample (*n*: 283) 194 SNPs were genotyped covering 19 candidate clock genes. In the confirmation

sample, two of the best association signals as analyzed by linear regression model were examined<sup>[16]</sup>. Associations are found in a *CLOCK* gene intronic region (rs12649507 and rs11932595). Significance persisted for the multiple-marker association signal of rs1264905/rs11932595 haplotype GGAA with long sleep. The authors surmised that an association exists between human *CLOCK* gene variants and sleep duration.

## SLEEP IN HUNTER-GATHERERS

One can hypothesize that before the advent of the electric bulb and the concept of shift-work, humans slept at sunset and awoke at sunrise, but evidence is hard to come by. A recent study on societies from Tanzania, Namibia and Bolivia, who are hunter-gathers/horticulturalists has provided information on their sleep pattern. These communities do not have access to electric light, television internet, nor do they use caffeine beverages. The principal findings are that their sleep duration averaged 6.9-8.5 h, with variation occurring due to changes in going to sleep, rather than their wake up time. Interestingly they slept on an average, 3.3 h after sunset, but generally woke up before sunrise<sup>[17]</sup>. Environmental temperature played a major part in regulating sleep, with falling temperatures associated with sleep. It is intriguing to consider whether temperature control in industrialized societies could be contributing, at least partly, to the disturbances of the sleep cycle.

In addition comparative analyses across species is possible by studying the genomic changes in the visual and olfactory ability of the kiwis<sup>[18]</sup>. Sequencing of the kiwi genome provided information about evolutionary changes in genomic sequences that allowed it to adopt to a nocturnal lifestyle.

## BURDEN OF DISEASE

Studying the global burden of acute and chronic diseases between 1990 and 2013 from 188 countries, non-communicable diseases were responsible for leading chronic sequelae<sup>[19]</sup>. Long working hours (defined as working more than 55 h/wk) were associated with increased risk of cerebrovascular disease<sup>[20]</sup>. The association with coronary artery disease was weaker; the strength of association with cerebrovascular disease was greater.

Type 2 diabetes mellitus, obesity and metabolic syndrome are known predisposing factors to vascular disease, both cardiovascular and cerebrovascular. Longer working hours entail both exposure to greater stress and a potential abbreviation of sleep duration and quality.

## SLEEP STUDIES: ORIGIN AND PROGRESS

Sleep has evolved from being considered a single uniform state<sup>[21]</sup>. However, epidemiological studies of

sleep disturbances appeared from the 1980's. Interest arose initially from sleep problems being associated with accidents and errors of human performance; in addition they were common, likely to increase in number, and recognition that sleep problems had immediate and long term consequences such as risk of premature death, cardiovascular disease, hypertension, inflammation, insulin resistance, type 2 diabetes and psychiatric disorders<sup>[22]</sup>.

While short sleep duration and long sleep duration had greater risk of developing type 2 diabetes, the Whitehall study evaluated whether a change in duration of sleep altered the risk of incident diabetes mellitus. Computation of sleep duration was made at four cycles of 5-years each: 1985-1988 to 1991-1994, 1991-1994 to 1997-1999, 1997-1997-1999 to 2002-2004 and 2002-2004 to 2007-2009. When compared to those who persistently slept 7 h, an increase of sleep of 2 h or more per night was associated with increasing risk of diabetes; similar increased risk was also observed in those who had persistent short duration of sleep. This is new evidence that individuals whose duration of sleep increased over time could be at risk of type 2 diabetes mellitus, which may be related in part, to weight gain<sup>[23]</sup>. The concept arises that sleep duration and disease risk must be interpreted in light of potential confounding factors such as physical debility. What is evident is that otherwise healthy adults do not habitually extend their sleep duration beyond optimal levels<sup>[24]</sup>.

Meanwhile a meta-analysis of sleep duration and risk of type 2 diabetes mellitus showed a U-shaped relation between duration of sleep and the risk of developing T2DM<sup>[25]</sup>. Among 482502 subjects who were followed up for periods between 2.5 and 16 years, there were 18483 who developed incident diabetes. Lowest risk of diabetes was found among those who slept 7-8 h a day. In comparison pooled relative risk for T2DM was 1.09 for each 1-h shorter sleep duration among those who slept less than 7 h/d; it was 1.14 for each 1-h increase of sleep duration among those who slept longer. This underscores the fact that optimal sleep duration, viz neither less nor more, is important in delaying or even preventing the onset of type 2 diabetes mellitus<sup>[25]</sup>.

## MEDIATORS OF ADVERSE CONSEQUENCES

A coupled relation exists between circadian and metabolic systems<sup>[26]</sup>, known mechanisms postulated include hormonal and hedonic causes, alteration in cardiovascular autonomic reactivity, exposure to ambient light, and shift work<sup>[17]</sup>. The basic concordance of the internal physiological system with external environment results from a natural selection process. Recent evidence from a rodent model suggested that those with 24-h "resonant" rhythms lived longer and produced more litter than those whose rhythms were shortened by a mutation of circadian *Cklc* allele<sup>[27]</sup>. This could have important



consequences in abnormal work or lighting schedules.

Shift work is a more common cause of rhythmic misalignment in modern society, which is associated with adverse health consequences. It is associated with a misalignment of behavioural and environmental cycles relative to endogenous circadian system. Short-term misalignment of circadian rhythm led to adverse cardiovascular risk factors in healthy adults<sup>[28]</sup>. The mediators involved increased blood pressure during sleep, decreased cardiac vagal modulation, increased serum levels of interleukin-6, C-reactive protein, resistin and tumour necrosis factor- $\alpha$ <sup>[28]</sup>. A putative link between shift work and hypertension, inflammation and cardiovascular risk may exist.

The concept of a “sleep connectome” can help understand how transition among the various stages of sleep occurs: Vigilance, non-REM sleep and REM sleep. A population of neuronal populations in medial cells which expressed *Atoh1* in embryonic life may be important for switching between sleep stages non-REM and REM<sup>[29]</sup>.

## CLINICAL RELEVANCE

How do all these genomic and biochemical alterations translate into human disease? A variety of sleep disturbances have been shown to parallel an increasing prevalence of non communicable diseases, particularly obesity and type 2 diabetes. The interaction may occur through changes in hormones that mediate appetite, altered responses to metabolic signals by peripheral tissues as well as to changes in energy intake and expenditure<sup>[30]</sup>. Increased prevalence of sleep disturbances in type 2 diabetes has been recognized which can impair metabolic control, and must be corrected<sup>[31,32]</sup>.

## UNDERLYING MECHANISMS OF THE CIRCADIAN CLOCK INTERACTIONS

Recent evidence has thrown light on the underlying mechanism of circadian clock disturbances (Figure 1). An interesting observation links the coordination of a peripheral clock gene with pancreatic islet function and the etiology of T2DM<sup>[33]</sup>. Glucose induced secretion of insulin follows a circadian pattern, with transcriptional control over insulin secretory pathway<sup>[34]</sup>. A specific circadian clock which is found in the  $\beta$  cell of pancreas releases insulin which is dependent on the time of the day<sup>[35]</sup>.

The hepatic glucose output is also similarly regulated by a circadian rhythm<sup>[36]</sup>. An “inverse-variance weighted, fixed-effect meta-analysis of results of adjusted associations and interactions between dietary intake/sleep duration...” and variants on cardiometabolic traits was carried out from 15 cohort studies. Of the clock genes, known *MTNR1B* associations were seen with higher fasting glucose. Nominally significant interactions occurred with carbohydrate ingestion and *MTNR1B-rs1387153* for

fasting glucose. Of practical interest, lower carbohydrate ingestion and normal sleep were suggested to reduce adverse cardiometabolic traits resulting from circadian-related variants of the gene<sup>[37]</sup>.

## OTHER MECHANISMS, AND CLOCK DYSREGULATION IN OTHER REGIONS

As already mentioned, shift work rather than primary sleep loss, is the more prevalent sleep disturbance in modern societies. An experimental study mimicking shift work was carried out to evaluate changes of clock genes in the peripheral tissues at the epigenetic and transcriptional level<sup>[38]</sup>. A randomized 2-period, 2-condition, crossover clinical study was performed in 15 healthy men. With acute sleep deprivation, adipose tissue showed greater methylation in the promoter region of *CRY1* and in two promoter-interacting enhancer regions of *PER1*. In the skeletal muscle, there was a reduction in gene expression of *BMAL1* and of *CRY1*. Thus shift workers may have tissue specific alteration of clock genes which may mediate adverse health effects<sup>[38]</sup>.

Sub-chronic sleep restriction alters insulin sensitivity at the liver, the peripheral tissues and of substrate utilization. Fourteen subjects were recruited to a randomized crossover study. As expected, sub-chronic sleep restriction was associated with decreased whole body insulin sensitivity, and of peripheral insulin sensitivity<sup>[39]</sup>. There was a modest increase of stress hormones (cortisol, metanephrine and normetanephrine), along with fasting non esterified fatty acids (NEFAs) and  $\beta$ -hydroxy butyrate. This suggests that there was peripheral insulin resistance following sub-chronic sleep restriction, with contributions from elevated NEFAs, cortisol and metanephrines<sup>[39]</sup>; the latter increase lipolysis and NEFA levels, leading to insulin resistance.

Sleep can influence the sympathetic nervous system, which in turn affects not only the cardiovascular system, but also the  $\beta$  cells of the pancreas<sup>[40]</sup>. Tasali *et al.*<sup>[41]</sup> reported that even three nights of disrupted slow wave sleep impaired glucose clearance after a glucose load due to sympathetic dominance. Both environmental and genetic polymorphisms can result in disturbances in sympathetic activity and slow wave sleep.

It is well known that sleep homeostasis is undisturbed in young women during their menstrual cycles. Because adverse metabolic effects begin in the peri-menopausal women, EEG patterns of women in mid-life were assessed in the laboratory (20 women in the early menopausal transition) and were compared with 11 women having insomnia. The study was performed in the follicular and luteal phase of the menstrual cycle. Both groups had more awakenings and a low percentage of slow wave sleep<sup>[42]</sup>. Midlife women, whether or not they were insomniac, had greater sleep disruption in the luteal phase, attributed to the effect of progesterone affecting the sleep regulatory circuits.

Another interesting mechanism for artificial light

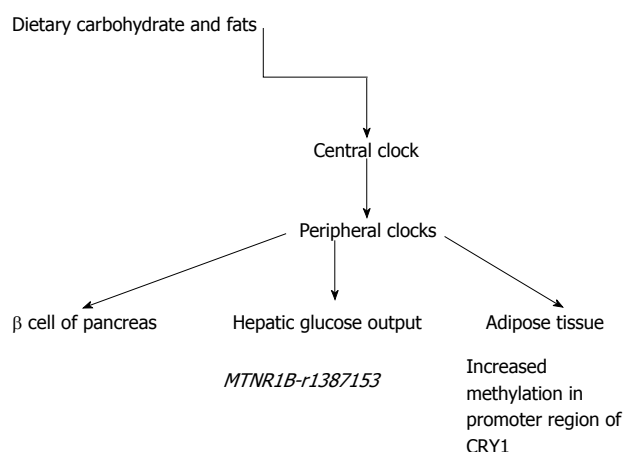


Figure 1 Newer players in circadian clock interactions.

induced obesity has been proposed: Disruption of the central clock mechanism can induce obesity by decreasing the energy expenditure. By increasing the number of hours exposed to light, attenuated brown adipose tissue activity increased body fat<sup>[43]</sup>. Prolonged light exposure reduces the sympathetic stimulation of brown adipose tissue and the  $\beta$ 3-adrenergic intracellular signal. These lower the uptake of fatty acids from triglyceride-rich lipoproteins, and of plasma glucose by brown adipose tissue<sup>[43]</sup>.

How do all these translate clinically? At baseline (year 2000), the Nurses' Health Study, recruited 59031 women without diabetes. On follow up until 2012, decreases in duration of sleep was associated with adverse changes in physical activity and quality of diet<sup>[44]</sup>. Therefore lifestyle measures must also be prescribed in preventing obesity and diabetes. An animal study showed that circadian disruption synergizes with diet-induced obesity leading to pancreatic  $\beta$ -cell failure. Wild type Sprague Dawley rats and *Period-1* luciferase reported transgenic rats were studied for 10 wk. Circadian disruption by continuous exposure to constant light acted together with diet-induced obesity to  $\beta$ -cell failure; the proposed mechanism was impaired function of the pancreatic islet clock function *via* impaired amplitude phase and inter-islet synchrony of clock transcriptional oscillation<sup>[45]</sup>.

In women of perimenopausal age, reproductive hormones influence physiological sleep. Thirty three perimenopausal women underwent a cross-sectional lab study for assessing interaction between sleep and reproductive hormones. Seventeen reported no sleep complaints while 16 had clinical insomnia. In the group without sleep complaints, follicular stimulating hormone (FSH) was positively associated with wakefulness after sleep onset and number of awakenings and arousals; the latter were defined using polysomnography<sup>[46]</sup>. On the contrary among those with known insomnia, sleep was correlated with anxiety and depression, but not with FSH level.

Iron may be another dietary regulator of circadian hepatic glucose metabolism. Little information is available

about the specific dietary agents that can influence hepatic glucose output. In an experimental method to assess the effect of iron in diet on circadian gluconeogenesis, dietary iron affected circadian glucose metabolism<sup>[47]</sup>. Iron modulates peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1 alpha), which affects hepatic heme through transcriptional activator of aminolevulinic acid synthase 1. Iron has a pivotal role in circadian rhythmicity through being bound to many circadian transcription factors. The levels of hepatic iron were kept within the physiological limits to avoid the known adverse effects of pathological hepatic overload as in hemochromatosis. Higher (physiological range) intake of iron altered the circadian rhythm of glucose and gluconeogenesis mediated through oxidative stress<sup>[48]</sup>.

In addition to iron, dietary fat and carbohydrate content also influence human clock genes. To clarify whether common dietary components can influence circadian rhythms, diurnal patterns of clock and other genes were studied in 29 non-obese healthy subjects. A baseline and one and six week switch of diets was studied (high carbohydrate-low fat diet and low carbohydrate-high fat isocaloric diet)<sup>[49]</sup>. Salivary cortisol showed a phase delay one and six weeks after dietary switch. Alterations were found in core clock genes by this switch (*PER1*, *PER2*, *PER3* and *TEF*) along with inflammatory genes (*CD14*, *CD180*, *NFKBIA*, *IL-1B*)<sup>[49]</sup>. Non-oscillating genes involved in energy and fat metabolism were also altered (*SIRT1*, *ACOX3*, *IDH3A*). Dietary carbohydrate and fat were thus shown to alter clock and other genes involved in energy metabolism (Table 1).

## SHIFT WORK

In the modern context, shift work is by far the most common cause for disturbed sleep and the consequent adverse health consequences. The effects may not be reversible, with persistent adverse cardiovascular outcomes documented on follow up<sup>[25]</sup>. As alluded to earlier, light-dark asynchronization accelerates weight gain in both animal models and in humans. The mechanistic explanations involved alteration in eating behavior, changes in hormones, alterations of melatonin, stress response due to lack of proper sleep. In addition recent evidence suggests that dysregulation of human transcriptome and metabolome could also contribute to adverse outcomes in shift workers<sup>[25]</sup>.

Another possible target in treatment strategies is the serotonin and serotonin transporter gene variant. Platelet 5-HT, 5-hydroxyindoleacetic acid (5-HIAA) and functional polymorphism of serotonin transporter gene (*SLC64A*) promoter were studied in rotating shift workers (*n*: 246) and in controls (*n*: 437 workers in day shift). There was a difference in platelet 5-HT between the two groups. 5-HIAA was higher in day workers<sup>[50]</sup>. Similar differences in genotype distribution were found in *SLCA4* promoter. It is possible to design drugs that can act at the serotonin pathway to manage adverse effects of shift work.

**Table 1** Other pathways influencing sleep

Stress hormones
Cortisol, metanephrine, normetanephrine
Sympathetic nervous system
Menstrual cycle
Decreased energy expenditure by artificial light
Reproductive hormones in women
Dietary iron

## SLEEP CHRONOTYPE

The concept of chronotype has been applied in humans to the onset of sleep. It is defined as a “construct that captures an individual's preference for being a ‘morning’ or ‘evening’ person”<sup>[51]</sup>. A recent study from Korea showed that at the level of population, an evening chronotype was associated with metabolic syndrome and diabetes, independent of other factors<sup>[52]</sup>. This was attributed to disturbed circadian rhythm impacting on metabolic regulation.

Improved work efficiency comes with the cost of adverse health outcomes, which must therefore be carefully balanced so that the risks do not outbalance the advantages<sup>[25]</sup>.

## SLEEP HYGIENE

Considering the overriding importance of adequate quality and quantity of sleep, a variety of ways have been devised to tackle this problem. They essentially involve avoiding stimulants at bedtime, proper sleep environment and in attempting to keep a regular sleep time<sup>[25]</sup>. In addition exercise if performed later in the day must be at least two hours before bedtime. Sleeping environment must be undisturbed, quiet, dark and comfortable.

## CONCLUSION

Sleep has multifactorial “macro” dimensions involving work and sleep hours, socioeconomic and health habits in addition to health<sup>[53]</sup>. Such cross disciplinary studies extend to interesting observation in black bears during hibernation, which conserve energy and bone mass. A reciprocal balance between bone resorption and formation during hibernation of bears was suggested to contribute to conservation of energy<sup>[54]</sup>. From a macro perspective, multilevel analyses in genomics have been proposed to study circadian rhythms in relation to mood<sup>[55]</sup>. Ultimately the concept of homeostasis has evolved from being a constant steady-state to a “constant steady rhythm”, linked by a network of mechanisms involving molecular clocks spanning gene transcription, metabolism, reproduction and behavior<sup>[55]</sup>. Establishment of this steady rhythm by balancing health vs productivity requires search further research. Currently it is a work in progress.

## REFERENCES

- 1 **Hazlerigg D**, Loudon A. New insights into ancient seasonal life timers. *Curr Biol* 2008; **18**: R795-R804 [PMID: 18786385 DOI: 10.1016/j.cub.2008.07.040]
- 2 **Mcnamara P**, Nunn CL, Barton RA. Introduction. In: McNamara P, Barton RA, Nunn CL, editors. *Evolution of sleep*. Cambridge: Cambridge Univ Press, 2010: 1-11
- 3 **Laposky A**, Easton A, Dugovic C, Walisser J, Bradfield C, Turek F. Deletion of the mammalian circadian clock gene BMAL1/Mop3 alters baseline sleep architecture and the response to sleep deprivation. *Sleep* 2005; **28**: 395-409 [PMID: 16171284]
- 4 **Turek FW**, Joshu C, Kohsaka A, Lin E, Ivanova G, McDearmon E, Laposky A, Losee-Olson S, Easton A, Jensen DR, Eckel RH, Takahashi JS, Bass J. Obesity and metabolic syndrome in circadian Clock mutant mice. *Science* 2005; **308**: 1043-1045 [PMID: 15845877 DOI: 10.1126/science.1108750]
- 5 **Turek FW**. Circadian clocks: tips from the tip of the iceberg. *Nature* 2008; **456**: 881-883 [PMID: 19092918 DOI: 10.1038/456881a]
- 6 **Foster RG**, Roenneberg T. Human responses to the geophysical daily, annual and lunar cycles. *Curr Biol* 2008; **18**: R784-R794 [PMID: 18786384 DOI: 10.1016/j.cub.2008.07.003]
- 7 **Sjodin A**, Hjorth MF, Damsgaard CT, Ritz C, Astrup A, Michaelsen KF. Physical activity, sleep duration and metabolic health in children fluctuate with the lunar cycle: science behind the myth. *Clin Obesity* 2015; **5**: 60-66 [DOI: 10.1111/cob.12117/full]
- 8 **Hughes AT**, Piggins HD. Disruption of daily rhythms in gene expression: the importance of being synchronised. *Bioessays* 2014; **36**: 644-648 [PMID: 24832865 DOI: 10.1002/bies.201400043]
- 9 **Sookoian S**, Castaño G, Gemma C, Gianotti TF, Pirola CJ. Common genetic variations in CLOCK transcription factor are associated with nonalcoholic fatty liver disease. *World J Gastroenterol* 2007; **13**: 4242-4248 [PMID: 17696255]
- 10 **Sookoian S**, Gemma C, Gianotti TF, Burgueño A, Castaño G, Pirola CJ. Genetic variants of Clock transcription factor are associated with individual susceptibility to obesity. *Am J Clin Nutr* 2008; **87**: 1606-1615 [PMID: 18541547]
- 11 **Gerhart-Hines Z**, Lazar MA. Circadian metabolism in the light of evolution. *Endocr Rev* 2015; **36**: 289-304 [PMID: 25927923]
- 12 **Froy O**. Metabolism and circadian rhythms--implications for obesity. *Endocr Rev* 2010; **31**: 1-24 [PMID: 19854863 DOI: 10.1210/er.2009-0014]
- 13 **Mariman EC**, Bouwman FG, Aller EE, van Baak MA, Wang P. Extreme obesity is associated with variation in genes related to the circadian rhythm of food intake and hypothalamic signaling. *Physiol Genomics* 2015; **47**: 225-231 [PMID: 25805767 DOI: 10.1152/physiolgenomics.00006.2015]
- 14 **Dashti HS**, Follis JL, Smith CE, Tanaka T, Cade BE, Gottlieb DJ, Hruby A, Jacques PF, Lamon-Fava S, Richardson K, Saxena R, Scheer FA, Kovanen L, Bartz TM, Perälä MM, Jonsson A, Frazier-Wood AC, Kalafati IP, Mikkilä V, Partonen T, Lemaître RN, Lahti J, Hernandez DG, Toft U, Johnson WC, Kanoni S, Raitakari OT, Perola M, Psaty BM, Ferrucci L, Grarup N, Highland HM, Rallidis L, Kähönen M, Havulinna AS, Siscovick DS, Rääkkönen K, Jørgensen T, Rotter JJ, Deloukas P, Viikari JS, Mozaffarian D, Linneberg A, Seppälä I, Hansen T, Salomaa V, Gharib SA, Eriksson JG, Bandinelli S, Pedersen O, Rich SS, Dedoussis G, Lehtimäki T, Ordovás JM. Habitual sleep duration is associated with BMI and macronutrient intake and may be modified by CLOCK genetic variants. *Am J Clin Nutr* 2015; **101**: 135-143 [PMID: 25527757 DOI: 10.3945/ajcn.114.095026]
- 15 **Garaulet M**, Esteban Tardido A, Lee YC, Smith CE, Parnell LD, Ordovás JM. SIRT1 and CLOCK > C combined genotype is associated with evening preference and weight loss resistance in a behavioral therapy treatment for obesity. *Int J Obes (Lond)* 2012; **36**: 1436-1441 [PMID: 22310473 DOI: 10.1038/ijo.2011.270]
- 16 **Allebrandt KV**, Teder-Laving M, Akyol M, Pichler I, Müller-Myhök B, Pramstaller P, Merrow M, Meitinger T, Metspalu A, Roenneberg T. CLOCK gene variants associate with sleep



- duration in two independent populations. *Biol Psychiatry* 2010; **67**: 1040-1047 [PMID: 20149345 DOI: 10.1016/j.biopsych.2009.12.026]
- 17 **Yetish G**, Kaplan H, Gurven M, Wood B, Pontzer H, Manger PR, Wilson C, McGregor R, Siegel JM. Natural sleep and its seasonal variations in three pre-industrial societies. *Curr Biol* 2015; **25**: 2862-2868 [PMID: 26480842 DOI: 10.1016/j.cub.2015.09.046]
  - 18 **Le Duc D**, Renaud G, Krishnan A, Almén MS, Huynen L, Prohaska SJ, Ongyerth M, Bitarello BD, Schiöth HB, Hofreiter M, Stadler PF, Prüfer K, Lambert D, Kelso J, Schöneberg T. Kiwi genome provides insights into evolution of a nocturnal lifestyle. *Genome Biol* 2015; **16**: 147 [PMID: 26201466 DOI: 10.1186/s13059-015-0711-4]
  - 19 **Global Burden of Disease Study 2013 Collaborators**. Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 2015; **386**: 743-800 [PMID: 26063472 DOI: 10.1016/S0140-6736(15)60692-4]
  - 20 **Kivimäki M**, Jokela M, Nyberg ST, Singh-Manoux A, Fransson EI, Alfredsson L, Bjorner JB, Borritz M, Burr H, Casini A, Clays E, De Bacquer D, Dragano N, Erbel R, Geuskens GA, Hamer M, Hooftman WE, Houtman IL, Jöckel KH, Kittel F, Knutsson A, Koskenvuo M, Lunau T, Madsen IE, Nielsen ML, Nordin M, Oksanen T, Peetersen JH, Pentti J, Rugulies R, Salo P, Shipley MJ, Siegrist J, Steptoe A, Suominen SB, Theorell T, Vahtera J, Westerholm PJ, Westerlund H, O'Reilly D, Kumari M, Batty GD, Ferrie JE, Virtanen M. Long working hours and risk of coronary heart disease and stroke: a systematic review and meta-analysis of published and unpublished data for 603,838 individuals. *Lancet* 2015; **386**: 1739-1746 [PMID: 26298822 DOI: 10.1016/S0140-6736(15)60295-1]
  - 21 **Lowy FH**. Recent sleep and dream research: clinical implications. *Can Med Assoc J* 1970; **102**: 1069-1077 [PMID: 4329501]
  - 22 **Ferrie JE**, Kumari M, Salo P, Singh-Manoux A, Kivimäki M. Sleep epidemiology--a rapidly growing field. *Int J Epidemiol* 2011; **40**: 1431-1437 [PMID: 22158659 DOI: 10.1093/ije/dyr203]
  - 23 **Ferrie JE**, Kivimäki M, Akbaraly TN, Tabak A, Abell J, Davey Smith G, Virtanen M, Kumari M, Shipley MJ. Change in Sleep Duration and Type 2 Diabetes: The Whitehall II Study. *Diabetes Care* 2015; **38**: 1467-1472 [PMID: 26068863 DOI: 10.2337/dc15-0186]
  - 24 **Stamatakis KA**, Punjabi NM. Long sleep duration: a risk to health or a marker of risk? *Sleep Med Rev* 2007; **11**: 337-339 [PMID: 17854737 DOI: 10.1016/j.smrv.2007.07.006]
  - 25 **Shan Z**, Ma H, Xie M, Yan P, Guo Y, Bao W, Rong Y, Jackson CL, Hu FB, Liu L. Sleep duration and risk of type 2 diabetes: a meta-analysis of prospective studies. *Diabetes Care* 2015; **38**: 529-537 [PMID: 25715415 DOI: 10.2337/dc14-2073]
  - 26 **Sridhar GR**, Lakshmi G. Sleep, obesity and diabetes: the circadian rhythm. In: Sridhar GR (Ed). *Advances in diabetes: newer insights*. New Delhi: The health Services Publisher, 2016: 196-207
  - 27 **Spoelstra K**, Wikelski M, Daan S, Loudon AS, Hau M. Natural selection against a circadian clock gene mutation in mice. *Proc Natl Acad Sci USA* 2016; **113**: 686-691 [PMID: 26715747 DOI: 10.1073/pnas.1516442113]
  - 28 **Morris CJ**, Purvis TE, Hu K, Scheer FA. Circadian misalignment increases cardiovascular disease risk factors in humans. *Proc Natl Acad Sci USA* 2016; **113**: E1402-E1411 [PMID: 26858430 DOI: 10.1073/pnas.1516953113]
  - 29 **Vyazovskiy VV**. Neuroscience. Mapping the birth of the sleep connectome. *Science* 2015; **350**: 909-910 [PMID: 26586746 DOI: 10.1126/science.aad6489]
  - 30 **Cedernaes J**, Schiöth HB, Benedict C. Determinants of shortened, disrupted, and mistimed sleep and associated metabolic health consequences in healthy humans. *Diabetes* 2015; **64**: 1073-1080 [PMID: 25805757 DOI: 10.2337/db14-1475]
  - 31 **Sridhar GR**, Madhu K. Prevalence of sleep disturbances in diabetes mellitus. *Diabetes Res Clin Pract* 1994; **23**: 183-186 [PMID: 7924879]
  - 32 **Surani S**, Brito V, Surani A, Ghamande S. Effect of diabetes mellitus on sleep quality. *World J Diabetes* 2015; **6**: 868-873 [PMID: 26131327 DOI: 10.4239/wjd.v6.i6.868]
  - 33 **Dibner C**, Schibler U. METABOLISM. A pancreatic clock times insulin release. *Science* 2015; **350**: 628-629 [PMID: 26542553 DOI: 10.1126/science.aad5412]
  - 34 **Perelis M**, Marcheva B, Ramsey KM, Schipma MJ, Hutchison AL, Taguchi A, Peek CB, Hong H, Huang W, Omura C, Allred AL, Bradfield CA, Dinner AR, Barish GD, Bass J. Pancreatic  $\beta$  cell enhancers regulate rhythmic transcription of genes controlling insulin secretion. *Science* 2015; **350**: aac4250 [PMID: 26542580 DOI: 10.1126/science.aac4250]
  - 35 **Yoo SH**, Yamazaki S, Lowrey PL, Shimomura K, Ko CH, Buhr ED, Slepka SM, Hong HK, Oh WJ, Yoo OJ, Menaker M, Takahashi JS. PERIOD2: LUCIFERASE real-time reporting of circadian dynamics reveals persistent circadian oscillations in mouse peripheral tissues. *Proc Natl Acad Sci USA* 2004; **101**: 5339-5346 [PMID: 14963227 DOI: 10.1073/pnas.0308709101]
  - 36 **Ando H**, Ushijima K, Shimba S, Fujimura A. Daily Fasting Blood Glucose Rhythm in Male Mice: A Role of the Circadian Clock in the Liver. *Endocrinology* 2016; **157**: 463-469 [PMID: 26653333 DOI: 10.1210/en.2015-1376]
  - 37 **Dashti HS**, Follis JL, Smith CE, Tanaka T, Garaulet M, Gottlieb DJ, Hruba A, Jacques PF, Kieft-de Jong JC, Lamon-Fava S, Scheer FA, Bartz TM, Kovanen L, Wojczynski MK, Frazier-Wood AC, Ahluwalia TS, Perälä MM, Jonsson A, Muka T, Kalafati IP, Mikkilä V, Ordovás JM. Gene-Environment Interactions of Circadian-Related Genes for Cardiometabolic Traits. *Diabetes Care* 2015; **38**: 1456-1466 [PMID: 26084345 DOI: 10.2337/dc14-2709]
  - 38 **Cedernaes J**, Osler ME, Voisin S, Broman JE, Vogel H, Dickson SL, Zierath JR, Schiöth HB, Benedict C. Acute Sleep Loss Induces Tissue-Specific Epigenetic and Transcriptional Alterations to Circadian Clock Genes in Men. *J Clin Endocrinol Metab* 2015; **100**: E1255-E1261 [PMID: 26168277 DOI: 10.1210/JC.2015-2284]
  - 39 **Rao MN**, Neylan TC, Grunfeld C, Mulligan K, Schambelan M, Schwarz JM. Subchronic sleep restriction causes tissue-specific insulin resistance. *J Clin Endocrinol Metab* 2015; **100**: 1664-1671 [PMID: 25658017 DOI: 10.1210/jc.2014-3911]
  - 40 **Dijk DJ**. Slow-wave sleep, diabetes, and the sympathetic nervous system. *Proc Natl Acad Sci USA* 2008; **105**: 1107-1108 [PMID: 18212114 DOI: 10.1073/pnas.0711635105]
  - 41 **Tasali E**, Leproult R, Ehrmann DA, Van Cauter E. Slow-wave sleep and the risk of type 2 diabetes in humans. *Proc Natl Acad Sci USA* 2008; **105**: 1044-1049 [PMID: 18172212 DOI: 10.1073/pnas.0706446105]
  - 42 **de Zambotti M**, Willoughby AR, Sassoon SA, Colrain IM, Baker FC. Menstrual Cycle-Related Variation in Physiological Sleep in Women in the Early Menopausal Transition. *J Clin Endocrinol Metab* 2015; **100**: 2918-2926 [PMID: 26079775 DOI: 10.1210/jc.2015-1844]
  - 43 **Kooijman S**, van den Berg R, Ramkisoensing A, Boon MR, Kuipers EN, Loeff M, Zonneveld TC, Lucassen EA, Sips HC, Chatzispyrou IA, Houtkooper RH, Meijer JH, Coomans CP, Biersmasz NR, Rensen PC. Prolonged daily light exposure increases body fat mass through attenuation of brown adipose tissue activity. *Proc Natl Acad Sci USA* 2015; **112**: 6748-6753 [PMID: 25964318 DOI: 10.1073/pnas.1504239112]
  - 44 **Cespedes EM**, Bhupathiraju SN, Li Y, Rosner B, Redline S, Hu FB. Long-term changes in sleep duration, energy balance and risk of type 2 diabetes. *Diabetologia* 2016; **59**: 101-109 [PMID: 26522276 DOI: 10.1007/s00125-015-3775-5]
  - 45 **Qian J**, Yeh B, Rakshit K, Colwell CS, Matveyenko AV. Circadian Disruption and Diet-Induced Obesity Synergize to Promote Development of  $\beta$ -Cell Failure and Diabetes in Male Rats. *Endocrinology* 2015; **156**: 4426-4436 [PMID: 26348474 DOI: 10.1210/en.2015-1516]
  - 46 **de Zambotti M**, Colrain IM, Baker FC. Interaction between reproductive hormones and physiological sleep in women. *J Clin Endocrinol Metab* 2015; **100**: 1426-1433 [PMID: 25642589 DOI: 10.1210/jc.2015-1516]



- 10.1210/jc.2014-3892]
- 47 **Simcox JA**, Mitchell TC, Gao Y, Just SF, Cooksey R, Cox J, Ajioka R, Jones D, Lee SH, King D, Huang J, McClain DA. Dietary iron controls circadian hepatic glucose metabolism through heme synthesis. *Diabetes* 2015; **64**: 1108-1119 [PMID: 25315005 DOI: 10.2337/db14-0646]
- 48 **Kalhan SC**, Ghosh A. Dietary iron, circadian clock, and hepatic gluconeogenesis. *Diabetes* 2015; **64**: 1091-1093 [PMID: 25805759 DOI: 10.2337/db14-1697]
- 49 **Pivovarova O**, Jürchott K, Rudovich N, Hornemann S, Ye L, Möckel S, Murahovschi V, Kessler K, Seltmann AC, Maser-Gluth C, Mazuch J, Kruse M, Busjahn A, Kramer A, Pfeiffer AF. Changes of Dietary Fat and Carbohydrate Content Alter Central and Peripheral Clock in Humans. *J Clin Endocrinol Metab* 2015; **100**: 2291-2302 [PMID: 25822100 DOI: 10.1210/jc.2014-3868]
- 50 **Sookoian S**, Gemma C, Gianotti TF, Burgueño A, Alvarez A, González CD, Pirola CJ. Serotonin and serotonin transporter gene variant in rotating shift workers. *Sleep* 2007; **30**: 1049-1053 [PMID: 17702275]
- 51 **Reutrakul S**, Hood MM, Crowley SJ, Morgan MK, Teodori M, Knutson KL, Van Cauter E. Chronotype is independently associated with glycemic control in type 2 diabetes. *Diabetes Care* 2013; **36**: 2523-2529 [PMID: 23637357 DOI: 10.2337/dc12-2697]
- 52 **Yu JH**, Yun CH, Ahn JH, Suh S, Cho HJ, Lee SK, Yoo HJ, Seo JA, Kim SG, Choi KM, Baik SH, Choi DS, Shin C, Kim NH. Evening chronotype is associated with metabolic disorders and body composition in middle-aged adults. *J Clin Endocrinol Metab* 2015; **100**: 1494-1502 [PMID: 25831477 DOI: 10.1210/jc.2014-3754]
- 53 **Blwise DL**. Invited commentary: cross-cultural influences on sleep-broadening the environmental landscape. *Am J Epidemiol* 2008; **168**: 1365-1366 [PMID: 18936435 DOI: 10.1093/aje/kwn336]
- 54 **McGee-Lawrence M**, Buckendahl P, Carpenter C, Henriksen K, Vaughan M, Donahue S. Suppressed bone remodeling in black bears conserves energy and bone mass during hibernation. *J Exp Biol* 2015; **218**: 2067-2074 [PMID: 26157160 DOI: 10.1242/jeb.120725]
- 55 **Li JZ**. Circadian rhythms and mood: opportunities for multi-level analyses in genomics and neuroscience: circadian rhythm dysregulation in mood disorders provides clues to the brain's organizing principles, and a touchstone for genomics and neuroscience. *Bioessays* 2014; **36**: 305-315 [PMID: 24853393 DOI: 10.1002/bies.201300141]

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## Basic Study

# Implanting 1.1B4 human $\beta$ -cell pseudoislets improves glycaemic control in diabetic severe combined immune deficient mice

Alastair D Green, Srividya Vasu, Neville H McClenaghan, Peter R Flatt

Alastair D Green, Neville H McClenaghan, Peter R Flatt, SAAD Centre for Pharmacy and Diabetes, University of Ulster, Coleraine, Northern Ireland BT52 1SA, United Kingdom

Srividya Vasu, Cell Growth and Metabolism Section, National Institute for Diabetes and Digestive and Kidney diseases, National Institutes of Health, Bethesda, MD 20892, United States

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**Correspondence to:** Srividya Vasu, PhD, Visiting Fellow, Cell Growth and Metabolism Section, National Institute for Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Old Georgetown Road and Center Drive, Bethesda, MD 20892, United States. [s.vasu@outlook.com](mailto:s.vasu@outlook.com)  
 Telephone: +1-301-4517001

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## Abstract

### AIM

To investigate the potential of implanting pseudoislets formed from human insulin-releasing  $\beta$ -cell lines as an alternative to islet transplantation.

### METHODS

In this study, the anti-diabetic potential of novel human insulin releasing 1.1B4  $\beta$ -cells was evaluated by implanting the cells, either as free cell suspensions, or as three-dimensional pseudoislets, into the subscapular region of severe combined immune deficient mice rendered diabetic by single high-dose administration of streptozotocin. Metabolic parameters including food and fluid intake, bodyweight and blood glucose were monitored throughout the study. At the end of the study animals were given an intraperitoneal glucose

tolerance test. Animals were then culled and blood and tissues were collected for analysis. Insulin and glucagon contents of plasma and tissues were measured by insulin radioimmunoassay and chemiluminescent enzyme-linked immunosorbance assay respectively. Histological analyses of pancreatic islets were carried out by quantitative fluorescence immunohistochemistry staining.

## RESULTS

Both pseudoislet and cell suspension implants yielded well vascularised  $\beta$ -cell masses of similar insulin content. This was associated with progressive amelioration of hyperphagia ( $P < 0.05$ ), polydipsia ( $P < 0.05$ ), body weight loss ( $P < 0.05$ ), hypoinsulinaemia ( $P < 0.05$ ), hyperglycaemia ( $P < 0.05 - P < 0.001$ ) and glucose tolerance ( $P < 0.01$ ). Islet morphology was also significantly improved in both groups of transplanted mice, with increased  $\beta$ -cell ( $P < 0.05 - P < 0.001$ ) and decreased alpha cell ( $P < 0.05 - P < 0.001$ ) areas. Whereas mice receiving 1.1B4 cell suspensions eventually exhibited hypoglycaemic complications, pseudoislet recipients displayed a more gradual amelioration of diabetes, and achieved stable blood glucose control similar to non-diabetic mice at the end of the study.

## CONCLUSION

Although further work is needed to address safety issues, these results provide proof of concept for possible therapeutic applicability of human  $\beta$ -cell line pseudoislets in diabetes.

**Key words:** Human  $\beta$ -cell line; 1.1B4; Cell therapy; Insulin; Pseudoislets

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**Core tip:** Human insulin-releasing 1.1B4  $\beta$ -cell suspensions and pseudoislets were implanted in streptozotocin-diabetic severe combined immune deficient mice to assess their antidiabetic potential. Both cell configurations yielded vascularised, insulin positive  $\beta$ -cell masses. These were associated with beneficial effects on hyperphagia, polydipsia, body weight, hypoinsulinaemia, hyperglycaemia and glucose tolerance. Both treatments were also associated with significant improvements in islet morphology and increased  $\beta$ : $\alpha$ -cell ratio. Pseudoislet recipients displayed gradual glucose normalization, while cell suspension recipients ultimately presented with hypoglycaemic complications. These results provide proof of concept for possible clinical artificial human  $\beta$ -cell pseudoislets, although further work is needed to address the tumourigenicity of clonal cell-lines.

Green AD, Vasu S, McClenaghan NH, Flatt PR. Implanting 1.1B4 human  $\beta$ -cell pseudoislets improves glycaemic control in diabetic severe combined immune deficient mice. *World J Diabetes* 2016; 7(19): 523-533 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v7/i19/523.htm> DOI: <http://dx.doi.org/10.4239/wjd.v7.i19.523>

## INTRODUCTION

Type 1 diabetes mellitus (T1DM) is caused by autoimmune mediated destruction of insulin producing  $\beta$ -cells in the pancreatic islets<sup>[1]</sup>. Uncontrolled hyperglycaemia leads to debilitating and in some cases life-limiting complications including retinopathy, nephropathy, neuropathy and metabolic ketoacidosis<sup>[2-5]</sup>. Protection against these ailments by insulin injections requires frequent monitoring of blood glucose to prevent over - or under-dosage. Hypoglycaemic episodes are not uncommon especially in brittle diabetes where patients often exhibit hypoglycaemia unawareness resulting in dangerous iatrogenic hypoglycaemia<sup>[6]</sup>. Cellular delivery of insulin achieved by replacement of pancreatic  $\beta$ -cells can help manage diabetes and in some cases eliminate the need for exogenous insulin therapy<sup>[7]</sup>.

At present, the two methods employed to replace lost  $\beta$ -cells in T1DM are pancreatic transplantation (PTx) and islet transplantation (ITx)<sup>[8]</sup>. PTx involves an invasive procedure performed in combination with kidney transplantation and necessitates chronic immunosuppression to prevent graft rejection<sup>[9,10]</sup>. In contrast, ITx represents a less invasive alternative to PTx where islets are isolated by enzymatic digestion of donor pancreata and then administered to the recipient by percutaneous infusion into the liver *via* the portal vein<sup>[8]</sup>. While less risky than whole organ transplantation, ITx is limited by the requirement for immunosuppression to prevent rejection and promote long-term islet graft functionality but the majority of patients still revert to insulin use within five years of treatment<sup>[11,12]</sup>. Nevertheless, ITx can provide temporary insulin independence and even partial graft function can prevent dangerous hypoglycaemic events<sup>[8,13,14]</sup>. Unfortunately, pancreatic donors are scarce and current practices often require use of islets from two or more separate donors. This practice is not practical on a large scale and so there is a great impetus to find alternative solutions especially given that implant function also frequently fails with time<sup>[8]</sup>.

One approach to providing a sustainable supply of insulin releasing tissue for transplantation is to generate insulin-producing cells from stem cells or to engineer cell-lines which mimic the functional response of normal human pancreatic  $\beta$ -cells<sup>[15-18]</sup>. Over the years, many rodent  $\beta$ -cell lines have been created by methods such as exposure of primary rodent  $\beta$ -cells to radiation or transfection with oncogenic viral vectors such as SV40<sup>[19-24]</sup>. While such cell-lines have proven invaluable in basic islet research their xenogeneic properties limit their therapeutic utility. Consequently, more recent endeavours have been focused on the creation of insulin-releasing cell-lines from human  $\beta$ -cells<sup>[25,26]</sup>. Unfortunately, this has proven to be extremely difficult as human  $\beta$ -cells tend to proliferate poorly and undergo rapid dedifferentiation when cultured *in vitro*. The majority of attempts to develop stable human  $\beta$ -cell lines have yielded cells with limited glucose sensitivity or insufficient insulin

content<sup>[27-32]</sup>.

Extensive functional studies using the novel human  $\beta$ -cell line 1.1B4 created by the electrofusion of freshly isolated human  $\beta$ -cells with immortal PANC1 epithelial partner cells have demonstrated that 1.1B4 cells possess intact cellular mechanisms for insulin production and secretion, and that they are responsive to glucose and other modulators of insulin secretion<sup>[25]</sup>. The cells also appear to possess similar cytoprotective mechanisms to primary  $\beta$ -cells<sup>[33-35]</sup>.

Like many  $\beta$ -cell-lines, 1.1B4 cells spontaneously form three dimensional pseudoislets after 5 to 7 d when grown in suspension culture. These pseudoislets are morphologically similar to isolated primary islets and show increased expression of cell-cell communication genes together with remarkable potentiation of insulin secretory responses to glucose and other secretagogues *in vitro*<sup>[25,36]</sup>. Moreover, 1.1B4 cells showed significantly enhanced resistance to cytotoxicity when configured as pseudoislets compared to monolayers<sup>[37]</sup>. Transplantation of cells configured as pseudoislets may represent an attractive model to improve graft survival, function and resistance to hyperglycaemia. In the present study the ability of human insulin secreting 1.1B4 cells, administered as single cell suspensions or pseudoislets, to rescue diabetes and restore blood glucose control was studied using severe combined immunodeficient (SCID) mice rendered diabetic by administration of streptozotocin (STZ). These immunodeficient mice were used to prevent rejection of human 1.1B4 cell implants.

## MATERIALS AND METHODS

### Cell culture and pseudoislet formation

The generation and characterisation of the human 1.1B4  $\beta$ -cell line has been described previously<sup>[25]</sup>. The cells were maintained at 37 °C with 5% CO<sub>2</sub> in RPMI-1640 media (Gibco® Invitrogen, Paisley, United Kingdom) containing 11.1 mol/L glucose and 2.0 mol/L L-glutamine supplemented with 10% (v/v) foetal calf serum (Gibco® Invitrogen, Paisley, United Kingdom) and antibiotics (100 U/mL penicillin and 0.1 g/L streptomycin) (Gibco® Invitrogen, Paisley, United Kingdom). Cells were given fresh media every 2-3 d as necessary and were routinely used from passage 25-35. The cell line is available to purchase from Sigma-Aldrich (Dorset, United Kingdom). To form pseudoislets, 1.1B4 cells were seeded at a density of  $1 \times 10^5$  cells/well into ultra-low-attachment, six-well, flat-bottomed plates (Corning Inc., NY, United States) with 5-mL/well culture medium. Cells typically formed three-dimensional pseudoislet clusters, each comprising 5000-6000 cells, within 5-7 d of seeding<sup>[37]</sup>.

### Animal and surgical procedures

Adult female SCID mice (15-20 wk) were bred and maintained under specific pathogen-free conditions in the Biomedical and Behavioral Research Unit (BBRU) at Ulster University, Coleraine. Food and water were provided *ad*

*libitum* unless specified otherwise. Diabetes was induced by intraperitoneal administration of streptozotocin (165 mg/kg) after an 8 h fast. Hyperglycaemia was controlled with intensive insulin therapy (15 mg/kg body weight intraperitoneal bovine insulin every 8 h) prior to and during the early engraftment period as indicated in the Figures. Suspensions of 1.1B4 cells ( $1 \times 10^7$  cells/mL) were administered in 500  $\mu$ L serum-free Roswell park memorial institute (RPMI) medium subscapularly into adipose tissue deposit at back of the neck using a 25-G needle. For pseudoislet implantation, harvested pseudoislets were resuspended at a density of 2000 pseudoislets per ml and 500  $\mu$ L was injected to the same location using an 18-G needle. Control mice received vehicle only. Food intake, water intake and body weight were monitored daily while blood glucose was measured once every 3 d using Ascensia contour glucose strips (Bayer, Uxbridge, United Kingdom). At the end of the study, glucose tolerance was determined by measuring blood glucose and plasma insulin levels after glucose administration (18 mmol/kg *bw i.p.*) at 0 and 15, 30, 60, 90 and 120 min. Finally, terminal blood samples were collected and implants and pancreata were collected for both histology and hormone content assessment. Timeline of the procedures is depicted in Figure 1. All animal procedures were performed in adherence to the United Kingdom home office regulations (United Kingdom Animal Scientific Procedures Act 1986) and "Principles of laboratory animal care" (NIH Publication no 86-23, revised 1985).

### Biochemical assays

Lysates of excised cell masses and pancreata were prepared by overnight extraction at 4 °C with acid ethanol (ethanol 75% v/v, water 23.5% v/v and concentrated HCl 1.5% v/v). Protein contents were determined by Bradford assay. Insulin was determined by radioimmunoassay as described previously<sup>[38]</sup>. Glucagon was determined using glucagon chemiluminescent assay (EZGLU-30K, Millipore, MA, United States) following manufacturer's instructions. Glucose in plasma samples was determined using an Analox GM9 glucose analyzer (Analox, London, United Kingdom).

### Immunohistochemistry

For peroxidase immunostaining, de-waxed and rehydrated sections were blocked in 0.3% (v/v) H<sub>2</sub>O<sub>2</sub> in 50% (v/v) methanol for 30 min to quench endogenous peroxidase activity, before incubation at 95 °C in citrate buffer (pH 6.0) for antigen retrieval. After cooling, sections were incubated at 4 °C with mouse anti insulin antibody (1:1000, Abcam, United Kingdom) overnight, and then incubated with ImmPRESS HRP anti mouse IgG (peroxidase) reagent (Vector labs, United Kingdom) and developed with 3, 3'-Diaminobenzidine substrate (Vector labs, United Kingdom). Lastly, sections were counterstained with haematoxylin at 60 °C for 5 min, and slides were cleared with Histo-clear II and mounted with Histomount



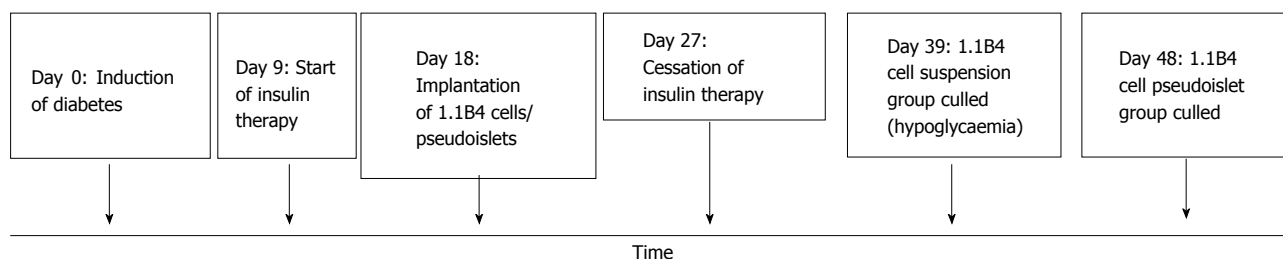


Figure 1 Timeline of experiment.

mounting medium. Slides were viewed using Olympus IX51 inverted microscope and photographed using the SPOT RT-Ke camera (Diagnostic Instruments Inc., Sterling Heights, MI, United States).

For fluorescence immunostaining, following dewaxing, rehydration, antigen retrieval with citrate buffer and blocking with BSA solution, sections were incubated at 4 °C overnight with primary antibodies (mouse anti insulin antibody, ab6995, 1:1000, Abcam; guinea pig anti glucagon antibody, PCA2/4, raised in house; rabbit anti Ki67 antibody, ab15580, 1:100, Abcam) prior to incubation at 37 °C for 45 min with secondary antibody (Alexa Fluor 488/594)<sup>[35,39]</sup>. Finally, slides were mounted with anti-fade mounting medium and viewed under FITC filter (488 nm) or TRITC filter using a fluorescent microscope (Olympus, model BX51) and photographed using a connected DP70 camera adapter system.

### Image analysis

Closed polygon tool in Cell-F image analysis software (Olympus Soft Imaging Solutions, GmbH) was used to analyze islet parameters including islet,  $\alpha$  cell and  $\beta$  cell areas. Number of islets was counted in a blinded fashion and expressed as number per mm<sup>2</sup> of pancreas. For analysis of islet size distribution, islets smaller than 10000  $\mu\text{m}^2$  were considered small, those larger than 10000  $\mu\text{m}^2$  but smaller than 25000  $\mu\text{m}^2$  were considered medium and those larger than 25000  $\mu\text{m}^2$  were considered large. Cells expressing both insulin and either Ki67 or TUNEL were counted and values were expressed as a percentage of the total number of insulin positive cells observed. Approximately 1000  $\beta$ -cells were analyzed per replicate.

### Statistical analysis

Results are expressed as mean  $\pm$  SEM. Groups of data were compared using Student's unpaired *t*-test with two-tailed *P*-values. Groups were considered significant where *P* < 0.05.

## RESULTS

### Effects on food and fluid intake, body weight and blood glucose

Streptozotocin diabetes caused significant increases in food and fluid intake when compared to non-diabetic controls (*P* < 0.05, *P* < 0.01, *P* < 0.001, Figure 2A and B).

Implantation of 1.1B4 cell suspensions or pseudoislets had small inhibitory effects on daily and cumulative food intake (Figure 2A). 1.1B4 pseudoislet transplantation significantly (*P* < 0.05) decreased fluid intake from day 18 post-implantation compared to the marked polydipsia exhibited by diabetic controls (Figure 2B). Fluid intake of cell suspension recipients did not significantly differ from control diabetic mice, indicating less effective amelioration of blood glucose control.

Streptozotocin diabetes resulted in significant and progressive body weight loss compared to non-diabetic controls (*P* < 0.05, *P* < 0.01, Figure 2C). Transplantation of 1.1B4 cells resulted in significantly increased body weight compared to diabetic controls 15 d post transplantation (*P* < 0.05, Figure 2C), while pseudoislets evoked a more gradual increase with values differing significantly from diabetic controls from 24 d post transplantation (*P* < 0.05, Figure 2C).

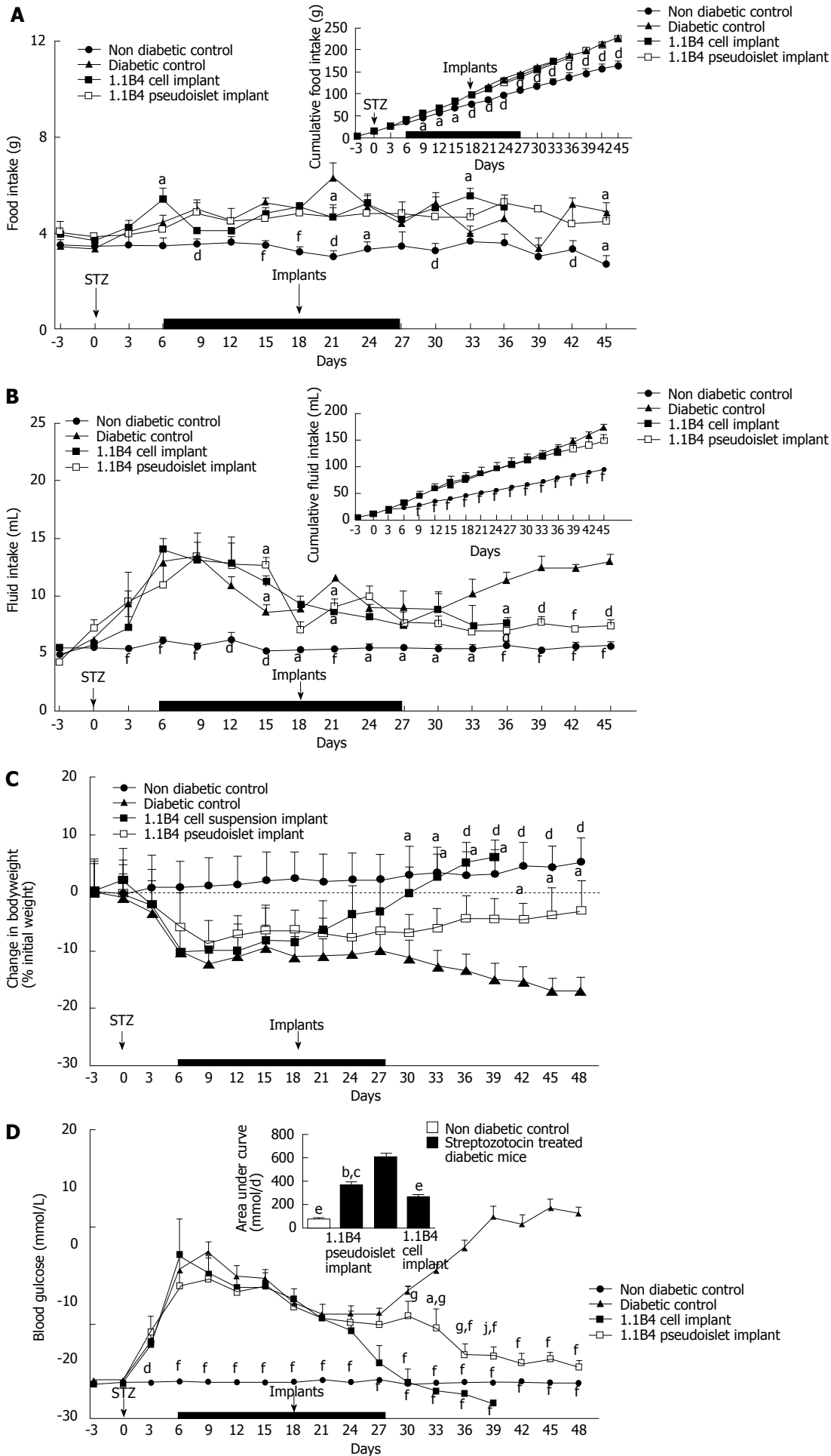
Streptozotocin diabetes significantly increased blood glucose levels within 3 d compared to non-diabetic controls (*P* < 0.001, Figure 2D). The hyperglycaemia was moderated during the period of insulin treatment but rebounded to very high levels thereafter. Blood glucose was significantly decreased at 12 and 15 d after implantation of 1.1B4 cells (*P* < 0.001, Figure 2D) or pseudoislets (*P* < 0.05, Figure 2D) respectively. From day 12 onwards, a much more moderate fall of blood glucose was observed in the pseudoislet recipient group (*P* < 0.05, *P* < 0.01, Figure 2D). Indeed, whereas mice receiving 1.1B4 cells were culled at 21 d post-transplantation to avoid severe hypoglycaemia, pseudoislet recipients exhibited normoglycaemia when the study was terminated at 30 d.

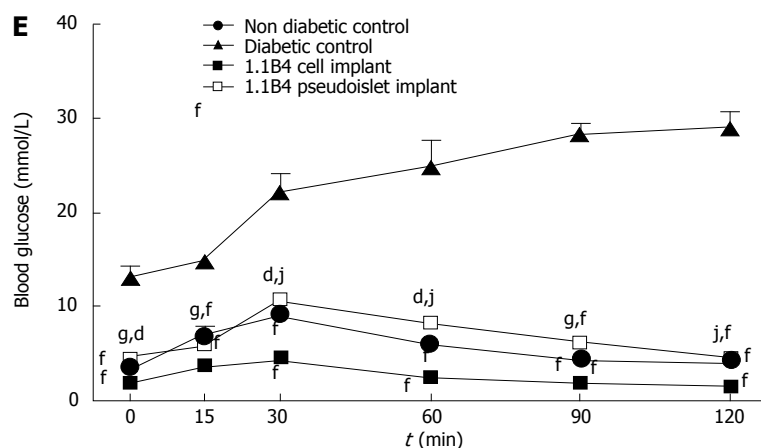
### Effects on glucose tolerance

Following an 8 h fast and intraperitoneal glucose administration, blood glucose levels of both 1.1B4 cell suspension and pseudoislet recipients were significantly lower than diabetic control animals at all time-points observed (*P* < 0.01, Figure 2E). Furthermore, 1.1B4 cell suspension implants yielded significantly (*P* < 0.05) lower blood glucose levels than pseudoislet implants or normal control mice (*P* < 0.05, *P* < 0.01, Figure 2E). Pseudoislet recipients exhibited normal glucose tolerance.

### Effects on plasma and pancreatic hormone content

Insulin content of cell suspension and pseudoislet implant





**Figure 2** Effects on food and fluid intake, body weight and blood glucose of streptozotocin diabetic severe combined immunodeficient mice implanted with 1.1B4 cells/ pseudoislets. A: Food intake; B: Fluid intake; C: Change in body weight; D: Blood glucose. From day 6-27, all diabetic mice were injected with insulin (15 U/kg bw) every 8 h (Indicated by black bar). At the end of the study, glucose tolerance (E) was determined over a time course of 120 min. Values are mean  $\pm$  SEM ( $n = 4$ ). <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$  and <sup>c</sup> $P < 0.001$  vs diabetic control animals; <sup>d</sup> $P < 0.05$ , <sup>e</sup> $P < 0.01$  vs 1.1B4 cell suspension recipients.

did not differ significantly (Figure 3A). Streptozotocin diabetes significantly decreased plasma insulin compared to non-diabetic mice ( $P < 0.001$ ). Insulin concentrations were significantly raised in mice receiving 1.1B4 cell suspension and pseudoislet implants (10.8 and 7.9 fold increases respectively,  $P < 0.05$ ,  $P < 0.01$ , Figure 3B). Streptozotocin diabetes also significantly decreased pancreatic insulin content ( $P < 0.05$ , Figure 3C) which was not altered by transplantation (Figure 3C). Plasma and pancreatic glucagon levels of diabetic mice were significantly increased compared to non-diabetic controls ( $P < 0.05$ ,  $P < 0.01$ , Figure 3D and E) and this was partly normalized by cell transplantation ( $P < 0.05$ , Figure 3D and E).

### Effects on pancreatic islets

Representative images showing insulin and glucagon staining in islets of non-diabetic, diabetic and cell/pseudoislet implanted diabetic mice are shown in Figure 4A. Histological analysis of the islets showed that streptozotocin markedly diminished islet area,  $\beta$  cell area,  $\beta$  to  $\alpha$  cell ratio and number of islets while increasing alpha cell area ( $P < 0.05$ ,  $P < 0.01$ ,  $P < 0.001$ , Figure 4B-F). Islet areas of 1.1B4 cell suspension recipients were marginally decreased compared to diabetic controls ( $P < 0.05$ , Figure 4B). However,  $\alpha$ -cell areas were decreased and both  $\beta$ -cell and  $\beta$ - to  $\alpha$ -cell ratios were significantly increased in 1.1B4 cell suspension and pseudoislet recipients ( $P < 0.05$ , Figure 4C-E). Percentage of smaller islets increased in diabetic mice which was not normalised by cell or pseudoislet transplantation (Figure 4G).

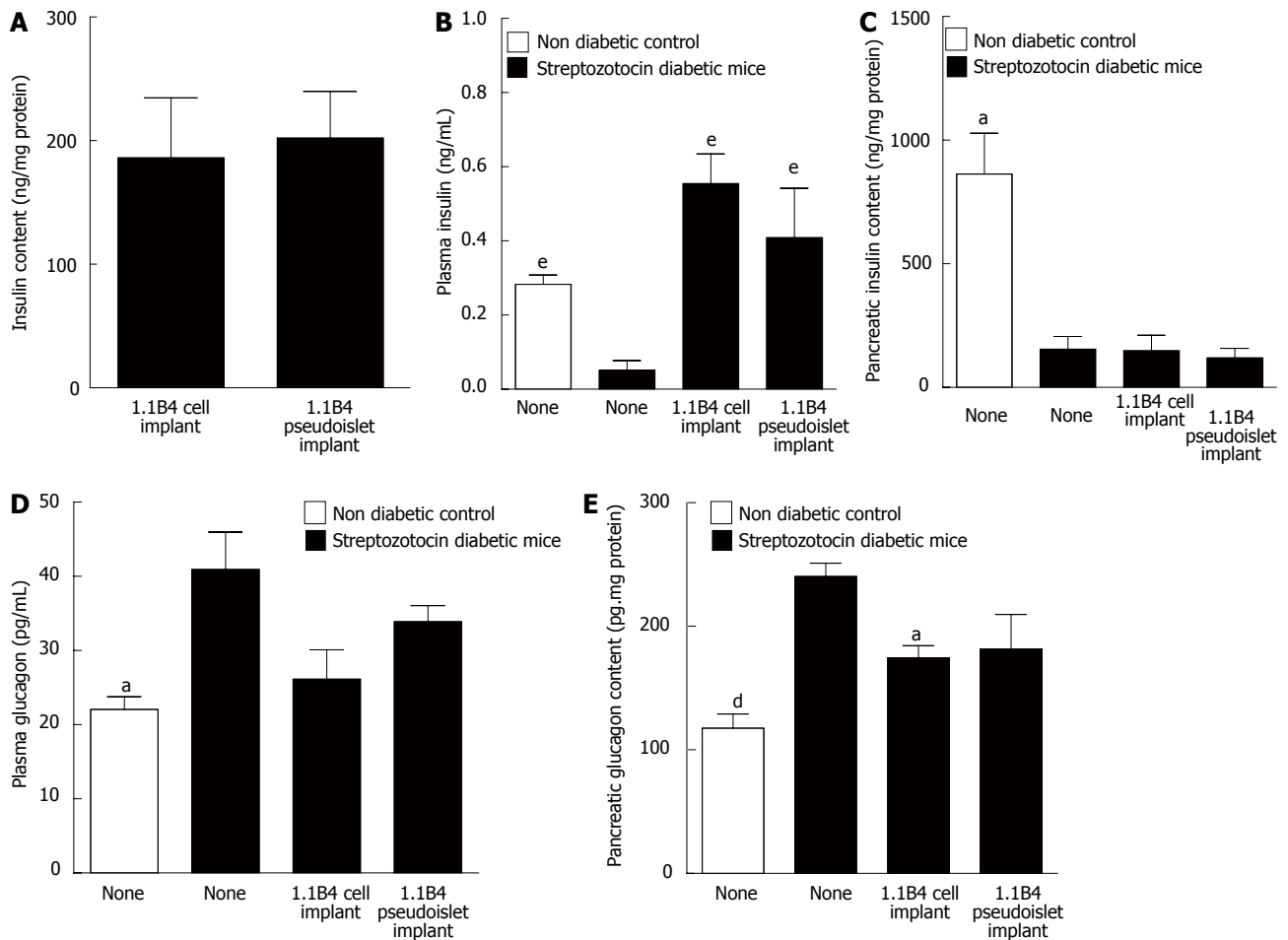
Representative images showing Ki67/insulin and TUNEL/insulin staining in islets of non-diabetic, diabetic and cell/pseudoislet implanted diabetic mice are shown in Figure 5A. Diabetes induction was associated with significant decreases in  $\beta$ -cell Ki67 to TUNEL ratio indicating an increase in the frequency of  $\beta$ -apoptosis and a decrease in  $\beta$ -cell proliferation ( $P < 0.05$ , Figure

5B-D). Implants did not significantly affect  $\beta$ -cell Ki67 or TUNEL expression.

## DISCUSSION

The therapeutic potential of novel 1.1B4 human insulin-releasing  $\beta$ -cells configured as cell suspensions or pseudoislets was assessed by implantation into diabetic SCID mice. 1.1B4 cells exhibit marked decreases in secretory function and viability following prolonged exposure to high levels of glucose<sup>[33,37]</sup>. As a result, mice with chemically-induced diabetes were given insulin therapy for 9-27 d after STZ to moderate blood glucose levels during the engraftment of implanted 1.1B4 cell suspensions and pseudoislets. As expected, control STZ-treated mice characteristically exhibited hyperphagia, polydipsia, weight loss and marked hyperglycaemia which were temporarily moderated during the period of insulin treatment.

Implantation of 1.1B4 cell suspensions or pseudoislets yielded vascularised cell masses (data not included) which restored plasma insulin concentrations and reversed the hyperglycaemic state. We did not have the opportunity to measure human C-peptide for confirmation but we assume that this insulin was derived from extra-pancreatic source because analysis of pancreatic tissue at end of study revealed severe loss of islet beta cells and cellular insulin in both 1.1B4 cell implanted groups similar to untreated diabetic controls. Furthermore, human insulin and C-peptide were readily detectable in 1.1B4 cells<sup>[25]</sup>. This was associated with significant beneficial effects on glucose tolerance, body weight and both, food and fluid intakes, but plasma glucagon remained elevated. These results have parallels with previous studies where primary islets were implanted into insulin controlled diabetic animals<sup>[40-42]</sup>. However, recipients of 1.1B4 cell suspensions progressed to low blood glucose levels such that these mice were terminated at 21 d after transplantation. In



**Figure 3** Insulin content (A) of excised 1.1B4 cell/pseudoislet cell masses and the effects of implantation on plasma insulin (B), pancreatic insulin content (C), plasma glucagon (D) and pancreatic glucagon content (E) of normal and streptozotocin diabetic severe combined immunodeficient mice. Values are mean  $\pm$  SEM ( $n = 4$ ). <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$ , <sup>c</sup> $P < 0.001$  vs diabetic control animals.

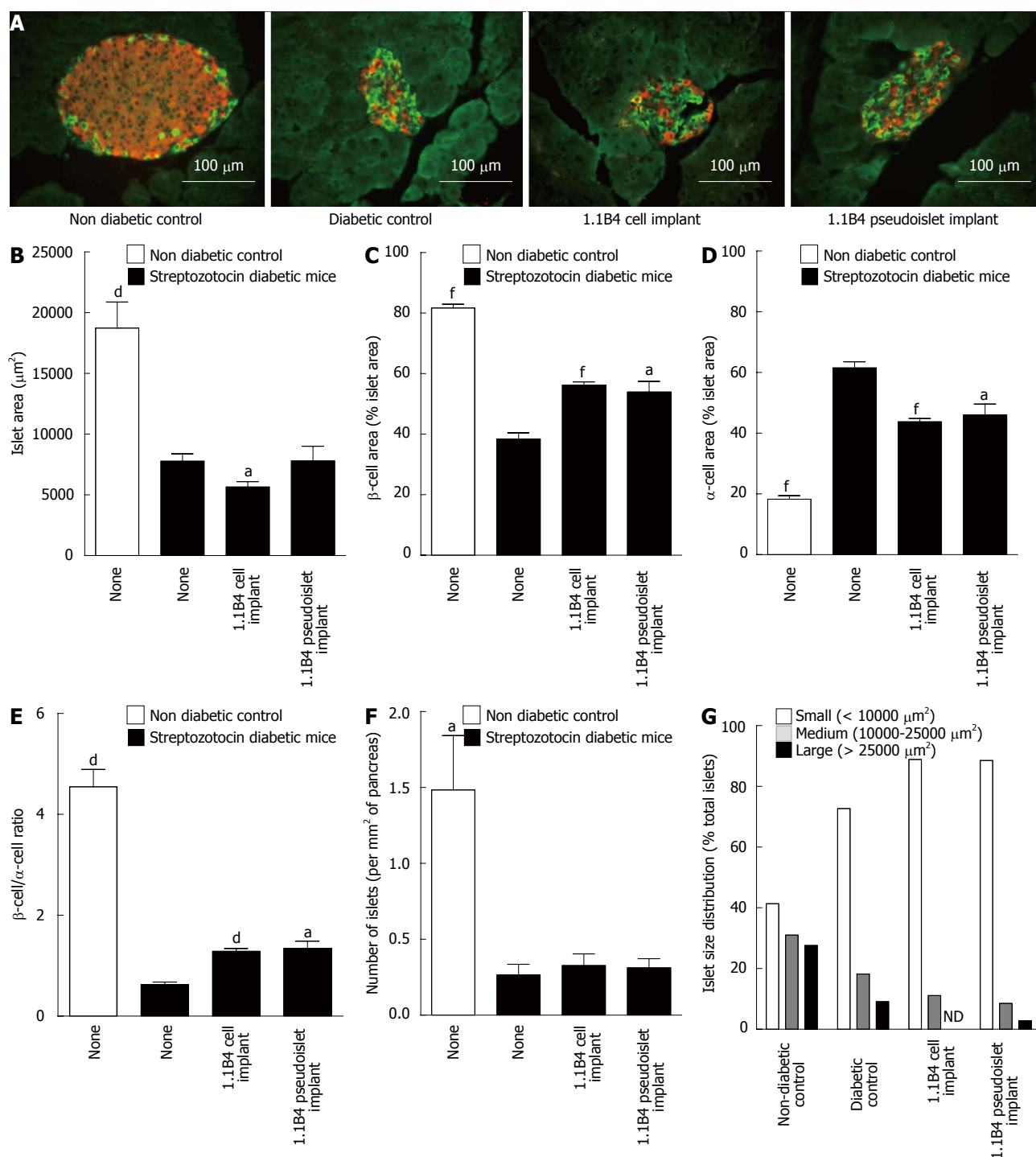
contrast, the anti-hyperglycaemic effects of pseudoislet implants manifested more slowly, achieving stable normoglycaemia without hypoglycaemic complications. Furthermore, energy and fluid balance, body weight, blood glucose and glucose tolerance improved gradually in these mice. This difference is most likely due to improved insulin secretory function in 1.1B4 pseudoislets compared to single isolated cells as described previously *in vitro*<sup>[25,36,37,43]</sup>. This better regulated insulin release is supported by similar insulin contents of the two types of resected  $\beta$ -cell masses. Nevertheless, part of the difference may also reflect the slower cellular proliferation following pseudoislet implantation.

Administration of STZ to SCID mice was associated with significant decreases in islet number, size and  $\beta$ -cell number together with significant  $\alpha$ -cell hyperplasia. These observations accompanied by depletion of pancreatic insulin and enhancement of pancreatic glucagon, mirror previous studies of animal models of diabetes induced by STZ<sup>[35,39,44-49]</sup>. Implantation of 1.1B4 cell suspensions did not affect hormone contents but was associated with decreases in  $\alpha$ -cell and islet areas but an increase in  $\beta$ -cell area and the  $\beta$ -cell to  $\alpha$ -cell ratio. There were no significant changes in  $\beta$ -cell proliferation

or apoptosis, so alterations of these processes in islet  $\alpha$ -cells merits further study. However, both pancreatic insulin and glucagon were unchanged in transplanted mice. Given the present interest in changes of  $\alpha$  cell populations in diabetes<sup>[35,47,49]</sup>, this observation merits further investigation. The effects on pancreatic hormones and islets were similar in pseudoislet recipients but as with the metabolic effects, they were moderate compared with cell suspension recipients.

Both cell suspensions and, to a lesser extent, 1.1B4 pseudoislets developed into cell masses following transplantation. While no obvious signs of metastasis were apparent in either group following post-mortem examination, the tumorigenic nature of the cells remains an obstacle to therapeutic use. 1.1B4 cells configured as pseudoislets exhibited significantly decreased proliferation rates and are self-limiting in size *in vitro*<sup>[36]</sup>. This might be a consequence of cell-cell contacts playing a role in modulation of proliferation and apoptosis rates. However, it seems likely that an additional factor limiting pseudoislet growth *in vitro* is hypoxia, a common consequence of culturing cell spheroids in static cultures. This view is supported by the ability of MIN6 mouse  $\beta$ -cell pseudoislets cultured in bioreactor with continuous



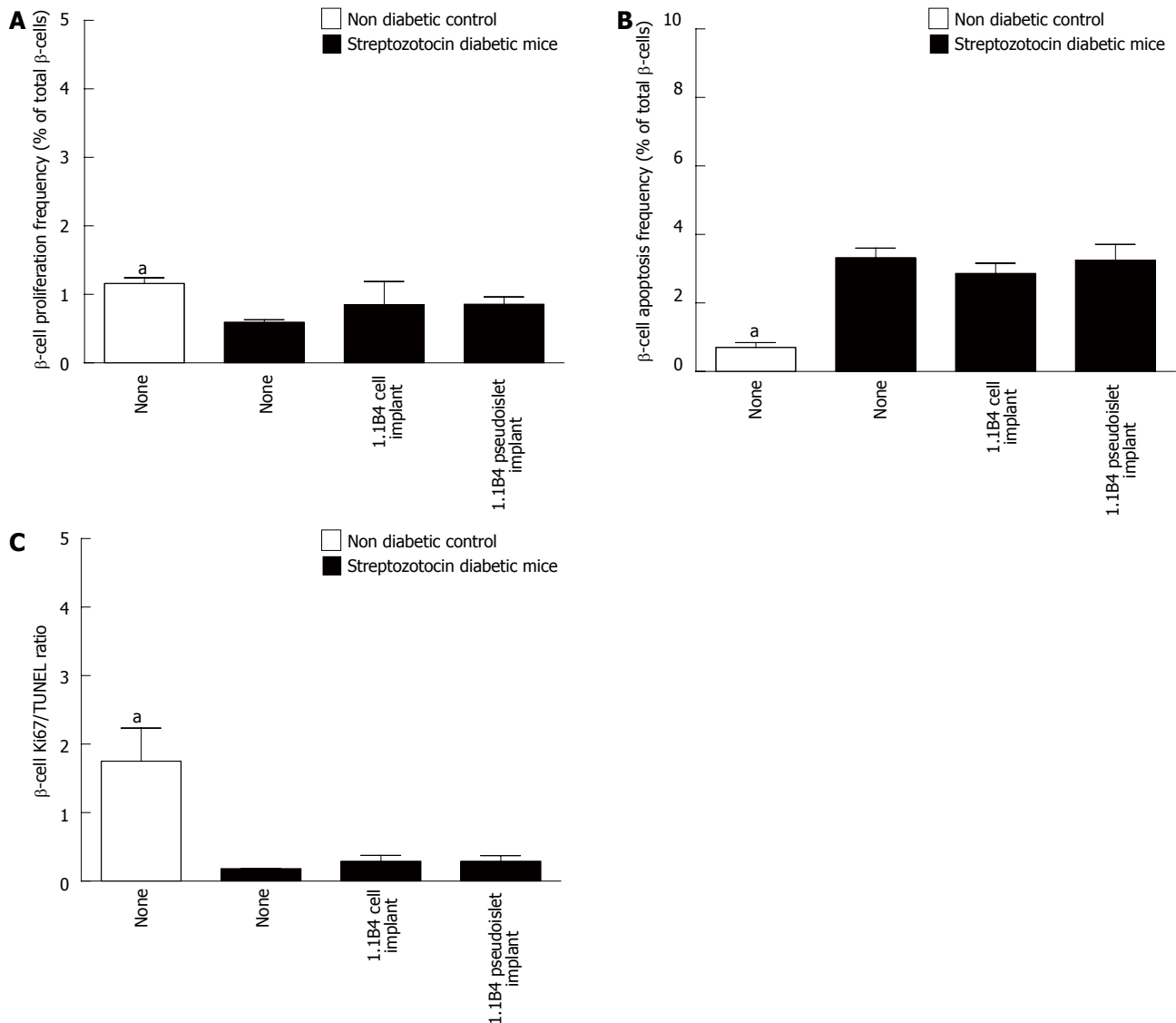


**Figure 4** Result of insulin (red) and glucagon (green) staining in islets of non-diabetic and diabetic severe combined immunodeficient mice with or without cell/pseudoislet transplantation. Representative images are shown in A. Islet area (B),  $\beta$  cell area (C),  $\alpha$  cell area (D),  $\beta$  to  $\alpha$  cell ratio (E), number of islets (F), and islet size distribution (G) were all determined by quantitative histological analysis using cell<sup>^</sup>F software. Values are mean  $\pm$  SEM ( $n = 5$ ). <sup>a</sup> $P < 0.05$ , <sup>d</sup> $P < 0.01$  and <sup>f</sup> $P < 0.001$  vs diabetic control.

stirring to grow continuously for two wk without exhibiting any signs of hypoxia, reduced functionality, or growth arrest<sup>[50]</sup>. *In vivo* 1.1B4 cells pseudoislets were able to quickly muster a blood supply which allowed proliferation of the cells. This contrasts with the limited ability of human islets to establish effective vascularisation which is a major hindrance to clinical islet outcomes<sup>[51]</sup>.

A number of groups have investigated potential

ways of getting around the issue of tumorigenicity of engineered  $\beta$ -cells which need to be generated in large numbers in culture. The most popular approach is the use of tailored viral vectors which allows the inactivation or excision of oncogenes from the cell-lines genomes to reverse the immortal status of the cells once enough have been generated for use<sup>[17,26,32]</sup>. If such an approach could potentially be tailored to reverse the tumorigenic status of 1.1B4 cells, the therapeutic qualities observed



**Figure 5** Frequency of β-cell proliferation (A) and apoptosis (B) and ratio of Ki67 to TUNEL positive β-cells (C) were determined by histological analysis. Values are mean ± SEM ( $n = 4$ ). Approximately 1000 β-cells were counted per replicate. <sup>a</sup> $P < 0.05$  vs diabetic controls.

in this study could be more usefully exploited for the treatment of T1DM. An additional or alternative approach involves the use of implantation devices that are currently under development<sup>[7,52]</sup>. These devices, such as TheraCyte™ macroencapsulation system and nanofiber-enabled encapsulation devices support cell function by providing good oxygen tension and protection from autoimmune attack, whilst providing against unwanted growth and spread of implanted cells<sup>[7,52,53]</sup>.

To conclude, implantation of human 1.1B4 cells configured as pseudoislets rescued diabetes and significantly improved glucose tolerance, providing stable blood glucose control. Although the results provide proof-of-concept for possible therapeutic use of genetically engineered human β-cells configured as pseudoislets, further work to circumvent the tumorigenic properties of the cells, by genetic manipulation using viral vectors or implantation devices, will be required before such an approach can be realised in a clinical setting.

## COMMENTS

### Background

The clinical practicality of anti-diabetic islet transplantation therapy is hampered by poor long-term graft survival and the limited availability of donor pancreata. Implanting bioengineered human insulin releasing β-cell lines could potentially provide unlimited cells for such therapy.

### Research frontiers

The electrofusion derived 1.1B4 human β-cell line has previously shown promise as a candidate for such therapy. Furthermore, *in vitro* studies of these cells have shown marked enhancements in functionality and survival when the cells were configured as pseudoislets rather than isolated cells.

### Innovations and breakthroughs

This is the first study to show that the implantation of 1.1B4 pseudoislets can reverse diabetes in an animal model and to demonstrate additional beneficial effects of such treatment on the endocrine pancreas.

### Applications

These results provide proof-of-concept for possible therapeutic use of

genetically engineered human  $\beta$ -cells configured as psuedoislets as an alternative to the unsustainable practice of implanting primary human islets.

### Peer-review

In this study, the authors investigated insulin secreting 1.1B4 cells as an option to rescue diabetes in severe combined immunodeficient mice. The manuscript is interesting, but several concerns need to be addressed before publication.

## REFERENCES

- 1 **Bluestone JA**, Herold K, Eisenbarth G. Genetics, pathogenesis and clinical interventions in type 1 diabetes. *Nature* 2010; **464**: 1293-1300 [PMID: 20432533 DOI: 10.1038/nature08933]
- 2 **Chiasson JL**, Aris-Jilwan N, Bélanger R, Bertrand S, Beauregard H, Ekoé JM, Fournier H, Havrankova J. Diagnosis and treatment of diabetic ketoacidosis and the hyperglycemic hyperosmolar state. *CMAJ* 2003; **168**: 859-866 [PMID: 12668546]
- 3 **Fong DS**, Aiello L, Gardner TW, King GL, Blankenship G, Cavallerano JD, Ferris FL, Klein R. Retinopathy in diabetes. *Diabetes Care* 2004; **27** Suppl 1: S84-S87 [PMID: 14693935 DOI: 10.2337/diacare.27.2007.s84]
- 4 **Aring AM**, Jones DE, Falko JM. Evaluation and prevention of diabetic neuropathy. *Am Fam Physician* 2005; **71**: 2123-2128 [PMID: 15952441]
- 5 **Gross JL**, de Azevedo MJ, Silveiro SP, Canani LH, Caramori ML, Zelmanovitz T. Diabetic nephropathy: diagnosis, prevention, and treatment. *Diabetes Care* 2005; **28**: 164-176 [PMID: 15616252 DOI: 10.2337/diacare.28.1.164]
- 6 **Vantyghem MC**, Press M. Management strategies for brittle diabetes. *Ann Endocrinol (Paris)* 2006; **67**: 287-296 [PMID: 17072232 DOI: 10.1016/s0003-4266(06)72600-2]
- 7 **Fotino N**, Fotino C, Pileggi A. Re-engineering islet cell transplantation. *Pharmacol Res* 2015; **98**: 76-85 [PMID: 25814189 DOI: 10.1016/j.phrs.2015.02.010]
- 8 **Onaca N**, Naziruddin B, Matsumoto S, Noguchi H, Klintmalm GB, Levy MF. Pancreatic islet cell transplantation: update and new developments. *Nutr Clin Pract* 2007; **22**: 485-493 [PMID: 17906273 DOI: 10.1177/0115426507022005485]
- 9 **Lam VW**, Pleass HC, Hawthorne W, Allen RD. Evolution of pancreas transplant surgery. *ANZ J Surg* 2010; **80**: 411-418 [PMID: 20618193 DOI: 10.1111/j.1445-2197.2010.05309.x]
- 10 **Gruessner RW**, Gruessner AC. The current state of pancreas transplantation. *Nat Rev Endocrinol* 2013; **9**: 555-562 [PMID: 23897173]
- 11 **Shapiro AM**, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, Kneteman NM, Rajotte RV. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 2000; **343**: 230-238 [PMID: 10911004 DOI: 10.1056/nejm200007273430401]
- 12 **Ryan EA**, Paty BW, Senior PA, Bigam D, Alfadhli E, Kneteman NM, Lakey JR, Shapiro AM. Five-year follow-up after clinical islet transplantation. *Diabetes* 2005; **54**: 2060-2069 [PMID: 15983207 DOI: 10.2337/diabetes.54.7.2060]
- 13 **McCall M**, Shapiro AM. Update on islet transplantation. *Cold Spring Harb Perspect Med* 2012; **2**: a007823 [PMID: 22762022 DOI: 10.1101/cshperspect.a007823]
- 14 **Chhabra P**, Brayman KL. Overcoming barriers in clinical islet transplantation: current limitations and future prospects. *Curr Probl Surg* 2014; **51**: 49-86 [PMID: 24411187 DOI: 10.1067/j.cpsurg.2013.10.002]
- 15 **Hohmeier HE**, Newgard CB. Cell lines derived from pancreatic islets. *Mol Cell Endocrinol* 2004; **228**: 121-128 [PMID: 15541576 DOI: 10.1016/j.mce.2004.04.017]
- 16 **Baiu D**, Merriam F, Odorico J. Potential pathways to restore  $\beta$ -cell mass: pluripotent stem cells, reprogramming, and endogenous regeneration. *Curr Diab Rep* 2011; **11**: 392-401 [PMID: 21800022 DOI: 10.1007/s11892-011-0218-7]
- 17 **Ravassard P**, Hazhouz Y, Pechberty S, Bricout-Neveu E, Armanet M, Czernichow P, Scharfmann R. A genetically engineered human pancreatic  $\beta$  cell line exhibiting glucose-inducible insulin secretion. *J Clin Invest* 2011; **121**: 3589-3597 [PMID: 21865645 DOI: 10.1172/JCI58447]
- 18 **Godfrey KJ**, Mathew B, Bulman JC, Shah O, Clement S, Gallicano GI. Stem cell-based treatments for Type 1 diabetes mellitus: bone marrow, embryonic, hepatic, pancreatic and induced pluripotent stem cells. *Diabet Med* 2012; **29**: 14-23 [PMID: 21883442]
- 19 **Santerre RF**, Cook RA, Crisel RM, Sharp JD, Schmidt RJ, Williams DC, Wilson CP. Insulin synthesis in a clonal cell line of simian virus 40-transformed hamster pancreatic beta cells. *Proc Natl Acad Sci USA* 1981; **78**: 4339-4343 [PMID: 6270673 DOI: 10.1073/pnas.78.7.4339]
- 20 **Hanahan D**. Heritable formation of pancreatic beta-cell tumours in transgenic mice expressing recombinant insulin/simian virus 40 oncogenes. *Nature* 1985; **315**: 115-122 [PMID: 2986015 DOI: 10.1038/315115a0]
- 21 **Ashcroft SJ**, Hammonds P, Harrison DE. Insulin secretory responses of a clonal cell line of simian virus 40-transformed B cells. *Diabetologia* 1986; **29**: 727-733 [PMID: 3026878 DOI: 10.1007/bf00870283]
- 22 **Miyazaki J**, Araki K, Yamato E, Ikegami H, Asano T, Shibasaki Y, Oka Y, Yamamura K. Establishment of a pancreatic beta cell line that retains glucose-inducible insulin secretion: special reference to expression of glucose transporter isoforms. *Endocrinology* 1990; **127**: 126-132 [PMID: 2163307 DOI: 10.1210/endo-127-1-126]
- 23 **Ishihara H**, Asano T, Tsukuda K, Katagiri H, Inukai K, Anai M, Kikuchi M, Yazaki Y, Miyazaki JI, Oka Y. Pancreatic beta cell line MIN6 exhibits characteristics of glucose metabolism and glucose-stimulated insulin secretion similar to those of normal islets. *Diabetologia* 1993; **36**: 1139-1145 [PMID: 8270128 DOI: 10.1007/bf00401058]
- 24 **Radvanyi F**, Christgau S, Baekkeskov S, Jolicœur C, Hanahan D. Pancreatic beta cells cultured from individual preneoplastic foci in a multistage tumorigenesis pathway: a potentially general technique for isolating physiologically representative cell lines. *Mol Cell Biol* 1993; **13**: 4223-4232 [PMID: 8391634 DOI: 10.1128/mcb.13.7.4223]
- 25 **McCluskey JT**, Hamid M, Guo-Parke H, McClenaghan NH, Gomis R, Flatt PR. Development and functional characterization of insulin-releasing human pancreatic beta cell lines produced by electrofusion. *J Biol Chem* 2011; **286**: 21982-21992 [PMID: 21515691 DOI: 10.1074/jbc.m111.226795]
- 26 **Scharfmann R**, Pechberty S, Hazhouz Y, von Bülow M, Bricout-Neveu E, Grenier-Godard M, Guez F, Rachdi L, Lohmann M, Czernichow P, Ravassard P. Development of a conditionally immortalized human pancreatic  $\beta$  cell line. *J Clin Invest* 2014; **124**: 2087-2098 [PMID: 24667639 DOI: 10.1172/jci72674]
- 27 **Thivolet C**, Chatelain P, Haftek M, Durand A, Pugeat M. [Morphologic and functional study of a human insulin-secreting cell line]. *C R Acad Sci III* 1986; **303**: 381-386 [PMID: 3022894 DOI: 10.1016/s0014-4827(85)80005-7]
- 28 **Gueli N**, Toto A, Palmieri G, Carmenini G, Delpino A. In vitro growth of a cell line originated from a human insulinoma. *J Exp Clin Cancer Res* 1987; **6**: 281-285
- 29 **Soldevila G**, Buscema M, Marini V, Sutton R, James RF, Bloom SR, Robertson RP, Mirakian R, Pujol-Borrell L, Bottazzo GF. Transfection with SV40 gene of human pancreatic endocrine cells. *J Autoimmun* 1991; **4**: 381-396 [PMID: 1680332 DOI: 10.1016/0896-8411(91)90154-5]
- 30 **Wang S**, Beattie GM, Mally MI, Cirulli V, Itkin-Ansari P, Lopez AD, Hayek A, Levine F. Isolation and characterization of a cell line from the epithelial cells of the human fetal pancreas. *Cell Transplant* 1997; **6**: 59-67 [PMID: 9040956 DOI: 10.1016/s0963-6897(96)00120-0]
- 31 **MacFarlane WM**, Chapman JC, Shepherd RM, Hashmi MN, Kamimura N, Cosgrove KE, O'Brien RE, Barnes PD, Hart AW, Docherty HM, Lindley KJ, Aynsley-Green A, James RF, Docherty K, Dunne MJ. Engineering a glucose-responsive human insulin-secreting cell line from islets of Langerhans isolated from a patient with persistent hyperinsulinemic hypoglycemia of infancy. *J Biol Chem* 1999; **274**: 34059-34066 [PMID: 10567373 DOI: 10.1074/jbc.274.48.34059]

- 32 **Narushima M**, Kobayashi N, Okitsu T, Tanaka Y, Li SA, Chen Y, Miki A, Tanaka K, Nakaji S, Takei K, Gutierrez AS, Rivas-Carrillo JD, Navarro-Alvarez N, Jun HS, Westerman KA, Noguchi H, Lakey JR, Leboulch P, Tanaka N, Yoon JW. A human beta-cell line for transplantation therapy to control type 1 diabetes. *Nat Biotechnol* 2005; **23**: 1274-1282 [PMID: 16186810 DOI: 10.1038/nbt1145]
- 33 **Vasu S**, McClenaghan NH, McCluskey JT, Flatt PR. Cellular responses of novel human pancreatic  $\beta$ -cell line, 1.1B4 to hyperglycemia. *Islets* 2013; **5**: 170-177 [PMID: 23985558 DOI: 10.4161/isl.26184]
- 34 **Vasu S**, McClenaghan NH, McCluskey JT, Flatt PR. Effects of lipotoxicity on a novel insulin-secreting human pancreatic  $\beta$ -cell line, 1.1B4. *Biol Chem* 2013; **394**: 909-918 [PMID: 23492555 DOI: 10.1515/hsz-2013-0115]
- 35 **Vasu S**, McClenaghan NH, McCluskey JT, Flatt PR. Mechanisms of toxicity by proinflammatory cytokines in a novel human pancreatic beta cell line, 1.1B4. *Biochim Biophys Acta* 2014; **1840**: 136-145 [PMID: 24005237 DOI: 10.1016/j.bbagen.2013.08.022]
- 36 **Guo-Parke H**, McCluskey JT, Kelly C, Hamid M, McClenaghan NH, Flatt PR. Configuration of electrofusion-derived human insulin-secreting cell line as pseudoislets enhances functionality and therapeutic utility. *J Endocrinol* 2012; **214**: 257-265 [PMID: 22685334]
- 37 **Green AD**, Vasu S, McClenaghan NH, Flatt PR. Pseudoislet formation enhances gene expression, insulin secretion and cytoprotective mechanisms of clonal human insulin-secreting 1.1B4 cells. *Pflugers Arch* 2015; **467**: 2219-2228 [PMID: 25559846]
- 38 **Flatt PR**, Bailey CJ. Abnormal plasma glucose and insulin responses in heterozygous lean (ob/+) mice. *Diabetologia* 1981; **20**: 573-577 [PMID: 7026332 DOI: 10.1007/bf00252768]
- 39 **Moffett RC**, Vasu S, Thorens B, Drucker DJ, Flatt PR. Incretin receptor null mice reveal key role of GLP-1 but not GIP in pancreatic beta cell adaptation to pregnancy. *PLoS One* 2014; **9**: e96863 [PMID: 24927416]
- 40 **Merino JF**, Nacher V, Raurell M, Aranda O, Soler J, Montanya E. Improved outcome of islet transplantation in insulin-treated diabetic mice: effects on beta-cell mass and function. *Diabetologia* 1997; **40**: 1004-1010 [PMID: 9300236 DOI: 10.1007/s001250050781]
- 41 **Ferrer-Garcia JC**, Merino-Torres JF, Pérez Bermejo G, Herrera-Vela C, Ponce-Marco JL, Piñon-Selles F. Insulin-induced normoglycemia reduces islet number needed to achieve normoglycemia after allogeneic islet transplantation in diabetic mice. *Cell Transplant* 2003; **12**: 849-857 [PMID: 14763504 DOI: 10.3727/000000003771000192]
- 42 **Kikawa K**, Sakano D, Shiraki N, Tsuyama T, Kume K, Endo F, Kume S. Beneficial effect of insulin treatment on islet transplantation outcomes in Akita mice. *PLoS One* 2014; **9**: e95451 [PMID: 24743240]
- 43 **Kelly C**, McClenaghan NH, Flatt PR. Role of islet structure and cellular interactions in the control of insulin secretion. *Islets* 2011; **3**: 41-47 [PMID: 21372635]
- 44 **Rahier J**, Goebbels RM, Henquin JC. Cellular composition of the human diabetic pancreas. *Diabetologia* 1983; **24**: 366-371 [PMID: 6347784]
- 45 **Clark A**, Wells CA, Buley ID, Cruickshank JK, Vanhegan RI, Matthews DR, Cooper GJ, Holman RR, Turner RC. Islet amyloid, increased A-cells, reduced B-cells and exocrine fibrosis: quantitative changes in the pancreas in type 2 diabetes. *Diabetes Res* 1988; **9**: 151-159 [PMID: 3073901 DOI: 10.1016/s0140-6736(87)90825-7]
- 46 **Li Z**, Karlsson FA, Sandler S. Islet loss and alpha cell expansion in type 1 diabetes induced by multiple low-dose streptozotocin administration in mice. *J Endocrinol* 2000; **165**: 93-99 [PMID: 10750039 DOI: 10.1677/joe.0.1650093]
- 47 **Yoon KH**, Ko SH, Cho JH, Lee JM, Ahn YB, Song KH, Yoo SJ, Kang MI, Cha BY, Lee KW, Son HY, Kang SK, Kim HS, Lee IK, Bonner-Weir S. Selective beta-cell loss and alpha-cell expansion in patients with type 2 diabetes mellitus in Korea. *J Clin Endocrinol Metab* 2003; **88**: 2300-2308 [PMID: 12727989 DOI: 10.1210/jc.2002-020735]
- 48 **Liu Z**, Kim W, Chen Z, Shin YK, Carlson OD, Fiori JL, Xin L, Napora JK, Short R, Odetunde JO, Lao Q, Egan JM. Insulin and glucagon regulate pancreatic  $\alpha$ -cell proliferation. *PLoS One* 2011; **6**: e16096 [PMID: 21283589 DOI: 10.1371/journal.pone.0016096]
- 49 **Meier JJ**, Ueberberg S, Korbas S, Schneider S. Diminished glucagon suppression after  $\beta$ -cell reduction is due to impaired  $\alpha$ -cell function rather than an expansion of  $\alpha$ -cell mass. *Am J Physiol Endocrinol Metab* 2011; **300**: E717-E723 [PMID: 21285404 DOI: 10.1152/ajpendo.00315.2010]
- 50 **Lock LT**, Laychock SG, Tzanakakis ES. Pseudoislets in stirred-suspension culture exhibit enhanced cell survival, propagation and insulin secretion. *J Biotechnol* 2011; **151**: 278-286 [PMID: 21185337]
- 51 **Liljeback H**, Grapensparr L, Olerud J, Carlsson PO. Extensive Loss of Islet Mass Beyond the First Day After Intraportal Human Islet Transplantation in a Mouse Model. *Cell Transplant* 2016; **25**: 481-489 [PMID: 26264975 DOI: 10.3727/096368915x688902]
- 52 **Kirk K**, Hao E, Lahmy R, Itkin-Ansari P. Human embryonic stem cell derived islet progenitors mature inside an encapsulation device without evidence of increased biomass or cell escape. *Stem Cell Res* 2014; **12**: 807-814 [PMID: 24788136]
- 53 **An D**, Ji Y, Chiu A, Lu YC, Song W, Zhai L, Qi L, Luo D, Ma M. Developing robust, hydrogel-based, nanofiber-enabled encapsulation devices (NEEDs) for cell therapies. *Biomaterials* 2015; **37**: 40-48 [PMID: 25453936 DOI: 10.1016/j.biomaterials.2014.10.032]

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## Basic Study

# Linagliptin alleviates fatty liver disease in diabetic *db/db* mice

Svetlana V Michurina, Irina Ju Ishenko, Vadim V Klimontov, Sergey A Archipov, Natalia E Myakina, Marina A Cherepanova, Eugenii L Zavjalov, Galina V Koncevaya, Vladimir I Konenkov

Svetlana V Michurina, Irina Ju Ishenko, Sergey A Archipov, Laboratory of Functional Morphology, Scientific Institute of Clinical and Experimental Lymphology, 630060 Novosibirsk, Russia

Vadim V Klimontov, Natalia E Myakina, Marina A Cherepanova, Laboratory of Endocrinology, Scientific Institute of Clinical and Experimental Lymphology, 630060 Novosibirsk, Russia

Eugenii L Zavjalov, Galina V Koncevaya, SPF-vivarium, Institute of Cytology and Genetics of Siberian Branch of Russian Academy of Sciences, 630090 Novosibirsk, Russia

Vladimir I Konenkov, Laboratory of Clinical Immunogenetics, Scientific Institute of Clinical and Experimental Lymphology, 630060 Novosibirsk, Russia

**Author contributions:** Michurina SV, Klimontov VV and Konenkov VI designed the research; Ishenko IJ, Myakina NE and Zavjalov EL conducted the experiments; Ishenko IJ, Archipov SA and Cherepanova MA performed the morphological investigations; Michurina SV, Ishenko IJ and Klimontov VV analyzed the data; Zavjalov EL and Koncevaya GV performed the biochemical investigations; Michurina SV, Ishenko IJ and Klimontov VV wrote the paper.

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**Institutional review board statement:** The protocol was approved by the Ethics Committee of Institute of Clinical and Experimental Lymphology (Protocol Number 1/2, April 1, 2014) and Inter-Institutional Animal Ethics Committee based on the Institute of Cytology and Genetics SB RAS (Permission Number: 21, April 1, 2014).

**Institutional animal care and use committee statement:** All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of the Russian National Center of Genetic Resources of Laboratory Animals based on the SPF Vivarium of the Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia (Permission Number:

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**Data sharing statement:** Statistical codes and the dataset are available from the corresponding author: klimontov@mail.ru.

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**Correspondence to:** Vadim V Klimontov, MD, PhD, Professor of Medicine, Deputy Director for Science, Head of the Laboratory of Endocrinology, Laboratory of Clinical Immunogenetics, Scientific Institute of Clinical and Experimental Lymphology, Timakov Street 2, 630060 Novosibirsk, Russia. klimontov@mail.ru  
Telephone: +7-913-9568299  
Fax: +7-383-3335122

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## Abstract

### AIM

To study the effects of linagliptin on the structural signs

of non-alcoholic fatty liver disease (NAFLD) in *db/db* mice.

### METHODS

Male diabetic *db/db* mice (BKS.Cg-Dock7<sup>m+</sup>/Lepr<sup>db</sup>/J) aged 10 wk received the dipeptidyl peptidase 4 (DPP4) inhibitor linagliptin (10 mg/kg) or saline as a placebo once per day by gavage for 8 wk. Intact *db/db* mice served as controls. Structural changes in the liver were analyzed from light and electron microscopic images of sections from intact, placebo-treated and linagliptin-treated animals. We estimated the changes in hepatocytes, sinusoidal cells, liver microvasculature and lymphatic roots. Hepatic staining for lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1) was assessed by immunohistochemistry.

### RESULTS

In 18-wk-old diabetic mice, liver steatosis (predominantly microvesicular and mediovesicular steatosis) was accompanied by dilation of the roots of the lymphatic system, interlobular blood vessels and bile canaliculi. Compared to saline-treated mice, linagliptin-treated mice exhibited a reduction in the mean numeral densities of hepatocytes with lipid droplets ( $92.4\% \pm 1.7\%$  vs  $64.9\% \pm 5.8\%$  per field of view,  $P = 0.0002$ ) and a lower proportion of hepatocytes with a high density of lipid droplets ( $20.7\% \pm 3.6\%$  vs  $50.4\% \pm 3.1\%$ ,  $P = 0.0007$ ). We observed heterogeneous hepatocytes and relatively preserved cell structures in the linagliptin group. Dilation of blood and lymphatic vessels, as well as ultrastructural changes in the hepatocyte endoplasmic reticulum and mitochondria, were alleviated by linagliptin treatment. In intact and placebo-treated mice, immunohistochemical staining for LYVE-1 was observed in the endothelial cells of interlobular lymphatic vessels and on the membranes of some endothelial sinusoidal cells. We observed an enlarged LYVE-1 reaction area in linagliptin-treated mice compared to intact and placebo-treated mice. The improvement in the structural parameters of the liver in linagliptin-treated mice was independent to changes in the plasma glucose levels.

### CONCLUSION

The DPP4 inhibitor linagliptin alleviates liver steatosis and structural changes in the hepatic microvasculature and lymphatic roots in a model of NAFLD in diabetic *db/db* mice.

**Key words:** Diabetes; Obesity; Non-alcoholic fatty liver disease; Dipeptidyl peptidase 4; Linagliptin

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**Core tip:** Dipeptidyl peptidase 4 (DPP4) inhibitors are a relatively new class of hypoglycemic agents with multiple pleiotropic effects. In this study, we demonstrated that the DPP4 inhibitor linagliptin alleviates liver steatosis and diminishes structural changes in hepatic non-parenchymal compartments in *db/db* diabetic mice. The mechanism

of the beneficial effect of linagliptin seems to be glucose-independent as no obvious hypoglycemic activity of the agent was observed in this model. The results of the study provide further evidence that linagliptin could be a promising agent for the treatment of non-alcoholic fatty liver disease in subjects with type 2 diabetes.

Michurina SV, Ishenko IJ, Klimontov VV, Archipov SA, Myakina NE, Cherepanova MA, Zavjalov EL, Koncevaya GV, Konenkov VI. Linagliptin alleviates fatty liver disease in diabetic *db/db* mice. *World J Diabetes* 2016; 7(19): 534-546 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v7/i19/534.htm> DOI: <http://dx.doi.org/10.4239/wjd.v7.i19.534>

### INTRODUCTION

Diabetes is associated with a spectrum of liver diseases, including non-alcoholic fatty liver disease (NAFLD) and steatohepatitis<sup>[1]</sup>. The current treatment for NAFLD primarily focuses on alleviating metabolic syndrome components *via* lifestyle modifications. However, the lack of success in their implementation and sustainment results in the need for effective pharmacological agents for the treatment of fatty liver<sup>[2]</sup>. Dipeptidyl peptidase 4 (DPP4) inhibitors are considered a new treatment option for NAFLD in patients with diabetes<sup>[3-5]</sup>. DPP4 inhibition reduces hepatic fat in experimental models of NAFLD<sup>[6-9]</sup>, but the underlying mechanisms remain to be clarified. Several clinical trials are exploring the efficacy of DPP4 inhibitors for the treatment of NAFLD<sup>[5,10-12]</sup>. DPP4 inhibitors might have a beneficial effect on hepatic steatosis and serum transaminase activity, but the data regarding the effects of DPP4 inhibitors on liver histology are scarce.

Although DPP4 inhibitors have the same mode of action, they differ by some important pharmacokinetic and pharmacodynamic properties that may be clinically relevant. Linagliptin is a highly specific, potent inhibitor of DPP4 that is currently indicated for the treatment of type 2 diabetes (T2D). In clinical studies, linagliptin effectively reduced glycated hemoglobin (HbA1c) levels in patients with T2D and exhibited a placebo-like safety and tolerability profile<sup>[13]</sup>. Linagliptin has an interesting pharmacokinetic profile in terms of its predominantly non-renal elimination. Fecal excretion is the dominant excretion pathway of linagliptin<sup>[14]</sup>. This DPP4 inhibitor is mainly excreted unchanged *via* bile, but is also excreted directly into the gut independent of biliary excretion<sup>[15]</sup>. Linagliptin also accumulates in hepatic tissue and exhibits both anti-inflammatory and anti-steatotic activity in a model of non-alcoholic steatohepatitis in streptozotocin-treated neonatal mice on a high-fat diet<sup>[8]</sup>. Long-term linagliptin treatment reduces liver fat content in mice with diet-induced hepatic steatosis and insulin resistance<sup>[6]</sup>.

Histopathological changes that occur with NAFLD are not limited by changes in the hepatic parenchyma. Involvement of other cell types (sinusoidal endothelial

cells, Kupffer cells, and stellate cells) and the recruitment of inflammatory cells and platelets lead to abnormal microcirculation and impaired intrahepatic fluid transport<sup>[16,17]</sup>. Despite the accumulating data on the favorable influence of DPP4 inhibitors on liver steatosis, the effects of these agents on non-parenchymal cells, bile transport, microcirculation and lymphatic drainage in the liver remain unknown. Therefore, we studied the long-term effects of the DPP4 inhibitor linagliptin on structural changes in hepatocytes, endothelial sinusoidal cells, and the interstitial compartments of the liver in *db/db* mice with obesity and T2D.

## MATERIALS AND METHODS

### Animal experiments

Twenty-four specific pathogen free (SPF) male *db/db* mice (BKS.Cg-*Dock7<sup>m</sup>/+Lepr<sup>db</sup>/J*) were utilized for the experiments. Mice homozygous for the diabetes spontaneous mutation (*Lepr<sup>db</sup>*) became identifiably polyphagic and obese at approximately 3 to 4 wk of age and exhibited elevated blood glucose from 4-8 wk. The animals were acclimatized to laboratory conditions for two weeks prior to experimentation. The mice were housed in individually ventilated cages (Animal Care Systems, Colorado, United States) in groups of one to four animals per cage with ad libitum food (Ssniff, Soest, Germany) and water. The mice were housed in a room within an SPF animal facility with a regular 14/10 h light/dark cycle (lights on 02:00 AM), a constant room temperature of 24 °C ± 2 °C, and a relative humidity of approximately 45% ± 10%.

After randomization, the experimental group of animals (*n* = 8) received linagliptin (Boeringer Ingelheim) at a dose of 10 mg/kg of body weight diluted in 200 µL of saline. Mice randomized to the "placebo" treatment (*n* = 8) received 200 µL of saline under the same scheme. Linagliptin or placebo was administered by gavage once per day for 56 d from the 10<sup>th</sup> to 18<sup>th</sup> week of age. Intragastric gavage administration was performed with conscious animals using straight gavage needles appropriate for the animal size. The control group was comprised of intact *db/db* male mice (*n* = 8).

At the 18<sup>th</sup> week, all mice were sacrificed by cervical dislocation under anesthesia. Liver samples were obtained for histological assessments, ultrastructural examinations and immunohistochemistry.

### Outcomes

All mice were weighed weekly during the experiment using electronic scales. Blood samples were obtained from the retro-orbital sinus of linagliptin-treated and placebo-treated mice at the 10<sup>th</sup>, 14<sup>th</sup> and 18<sup>th</sup> weeks. No stress-inducible procedures, including blood sample collections, were performed in intact animals. Blood samples were centrifuged to obtain plasma that was stored at -20 °C until analysis. The levels of glucose, triglycerides, total cholesterol, alanine aminotransferase

(ALT), and gamma-glutamyl transpeptidase (GGT) in the blood plasma were measured using automatic clinical chemistry system (Dade Behring Inc, United States) and commercially available cartridges according to the manufacturer's instructions (Dimension Clinical kit, Siemens, United States).

Liver samples for the light-optical studies were fixed in 10% formalin (pH = 7.4), dehydrated in alcohol at increasing concentrations and embedded in Histomix material (BioVitrum, Russia). Sections 3-4 microns thick were prepared on a microtome LEICA RM2155 (Germany, Switzerland) and were stained with Mayer's hematoxylin and eosin (H and E). Liver samples for electron microscopy were fixed in a 4% solution of paraformaldehyd with 0.1 mol/L phosphate buffer (PB, pH = 7.4) followed by 1% OsO<sub>4</sub>. The samples were then dehydrated and embedded in Epon-812. Using the LEICA TM UC7 ultratom (Germany), semi-thin sections (1 micron thick) were prepared and stained with toluidine blue. Liver sections 35-45 nm thick were contrasted with aqueous uranyl acetate solution and lead citrate and were studied with the JEOL JEM-1400 electron microscope (Japan).

A morphometric analysis of computed digital images of semi-thin sections from the livers of placebo-treated and linagliptin-treated mice was used to evaluate liver steatosis. Specifically, we calculated the proportion of hepatocytes containing lipid droplets and the distribution of hepatocytes with different lipid droplet densities. Hepatocytes were attributed to a cell population with a high density of lipid inclusions if more than 15 lipid droplets were revealed in the cytoplasm. Low lipid accumulation density was defined as hepatocytes containing less than five droplets. Microvesicular steatosis was defined by the presence of small cytoplasmic lipid droplets around a centrally positioned nucleus. Steatosis was considered mediovesicular when several medium-sized lipid vacuoles were present in the cytoplasm of the hepatocytes<sup>[18]</sup>. Macrovesicular steatosis was recorded when the diameter of the lipid droplets exceeded half of the hepatocyte nucleus diameter. We also calculated the numeral density of hepatocytes with different sized lipid droplets and estimated the proportions of cells with micro-sized, middle-sized and macro-sized lipid droplets in the cytoplasm. For cases in which the lipid droplets were of different sizes, each cell was taken into account twice or thrice.

Immunohistochemical detection of the lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1) marker was performed on 3-mm thick sections from the livers of intact, placebo-treated and linagliptin-treated mice using an indirect avidin-biotin ABC-peroxidase method with the VECTASTAIN Universal Quick Kit (Vector Laboratories, United States). Blocking of endogenous peroxidase was performed by incubating the sections in a 0.3% H<sub>2</sub>O<sub>2</sub> solution for 10 min with a subsequent incubation in normal horse non-immune blocking serum for 20 min. Next, the sections were incubated for one

**Table 1** Body weight and plasma biochemical parameters of the placebo-treated and linagliptin-treated *db/db* mice at the 10<sup>th</sup>, 14<sup>th</sup> and 18<sup>th</sup> week of age

Parameters	Placebo group ( <i>n</i> = 8)			Linagliptin group ( <i>n</i> = 8)		
	10 wk	14 wk	18 wk	10 wk	14 wk	18 wk
Body weight, g	35.1 (25.6-47.7)	33.2 (24.6-50.9)	37.3 (27.3-51.6)	37.6 (34.5-44.2)	39.9 (30.0-42.3)	41.7 (31.5-45.0)
Glucose, mg/dL	637 (549-678)	579 (551-671)	610 (506-683)	651 (631-693)	588 (520-640)	625 (601-646)
Triglycerides, mg/dL	415 (209-510)	324 (209-336)	316 (149-555)	385 (262-637)	279 (251-315)	391 (238-480)
Total cholesterol, mg/dL	129 (94-156)	100 (39-140)	131 (22-156)	112 (28-132)	104 (24-124)	71 (18-120)
ALT, U/L	132 (105-375)	126 (72-369)	170 (69-306)	146 (84-255)	185 (118-225)	203 (80-294)
GGT, U/L	16.7 (8.2-28.1)	13 (8.5-25.1)	14.6 (10.5-16.5)	14.4 (8.9-18.7)	12.5 (7.1-22)	13.6 (10.5-22.7)

Data are shown as the medians, minimal and maximal values. No significant differences in the variables in both groups at week 10 and 18 (Wilcoxon signed rank test, all  $P > 0.05$ ). The differences between groups are not significant at week 10, 14 and 18 ( $U$ -test, all  $P > 0.05$ ). ALT: Alanine aminotransferase; GGT: Gamma-glutamyl transpeptidase.

hour at room temperature with anti-LYVE-1 (Isotype: Rabbit polyclonal, bs-1311R; Bioss) at a final dilution of 5 mg/mL; washed in 3 changes of phosphate buffer for 3 min; and further incubated for 30 min at room temperature with a biotinylated second antibody followed by washing in 3 changes of phosphate buffer for 5 min. Incubation with the ABC-peroxidase complex was performed for 30 min at room temperature followed by washing in 3 changes of phosphate buffer for 5 min. Immunohistochemical staining of the sections was performed with a chromogenic substrate (ImmPACT DAB, Vector Laboratories, United States). To quantify the LYVE-1 staining, computed morphometric analysis of the digital images was performed using the "VideoTest Morpho 3.2" program.

### Ethical issues

All animal experiments were performed in compliance with the protocols and recommendations for the proper use and care of laboratory animals (ECC Directive 86/609/EEC). The protocol was approved by the Ethics Committee of Institute of Clinical and Experimental Lymphology (Protocol Number 1/2, April 1, 2014), and by the Inter-Institutional Animal Ethics Committee based on the Institute of Cytology and Genetics SB RAS (Permission Number: 21, April 1, 2014). All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of the Russian National Center of Genetic Resources of Laboratory Animals based on the SPF Vivarium of Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia (Permit Number: 246, April 8, 2014). All efforts were made to minimize the number of animals used and their pain or discomfort.

### Statistical analysis

Statistical processing of the results was performed using the STATISTICA software package 10 (StatSoft Inc., United States). A statistical review of the study was performed by a biomedical statistician. The Shapiro-Wilk test was used for testing normality. For the analysis of normally distributed quantitative data, the mean (M) and standard error of the mean (SEM) were calculated. The significance of differences between the groups was

assessed by Student's  $t$ -test. Non-normally distributed data (body weights and biochemical parameters) are presented as medians with minimum and maximum values; the significance of differences was determined using the non-parametric Mann-Whitney  $U$ -test or Wilcoxon signed rank test for repeated measurements. The differences were considered significant at  $P < 0.05$ .

## RESULTS

### Body weight and biochemical parameters

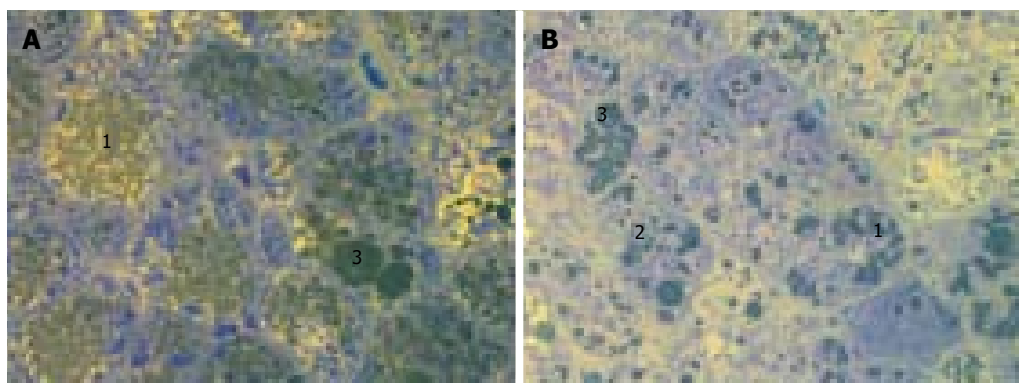
As expected, *db/db* mice became obese by week 10. The weight of the animals remained stable throughout experiment (Table 1). All animals had severe hyperglycemia at the 10<sup>th</sup> week with plasma glucose levels of 506 mg/dL (28.1 mmol/L) or more. The glucose levels remained elevated throughout the experiment in both the linagliptin and placebo groups. No significant differences in the levels of glucose, triglycerides, total cholesterol, ALT and GGT were observed between the groups at week 10, 14 or 18.

### Liver histology

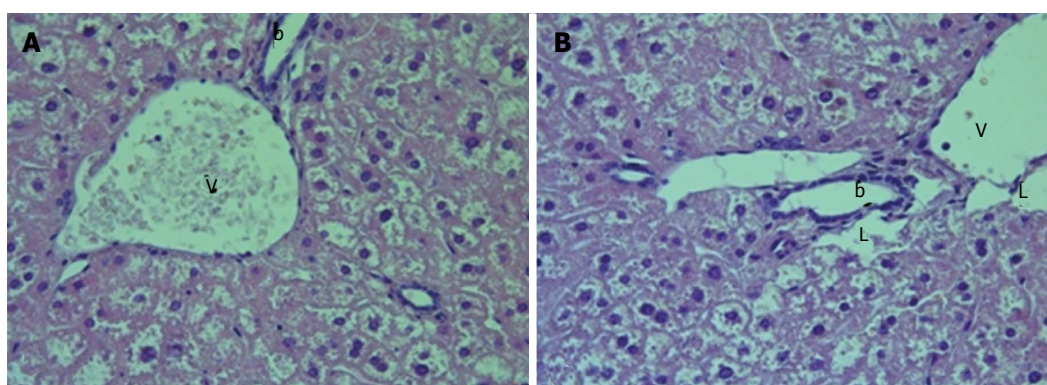
We observed diffuse lipid accumulation in the livers of all 18-wk-old *db/db* diabetic mice. Lipid droplets were found in  $92.4\% \pm 1.7\%$  of hepatocytes per field of view. Microvesicular and mediovesicular steatosis was the principal morphological finding, although sporadic large lipid droplets were also observed (Figure 1). Vacuolar degeneration was found in the pericentral and intermediate zones of predominantly hepatic lobuli. In some cells, glycogenized nuclei were noticed. The dilation of interlobular arteries and veins, central and sublobular veins, lymphatic vessels and bile canaliculi was present in most of the histological preparations (Figure 2). These changes were accompanied by edema in the connective tissue layers. The sludge of erythrocytes was found in intralobular sinusoidal capillaries. We detected no signs of inflammatory infiltration or interstitial fibrosis.

The liver histology in placebo-treated mice was very similar to intact animals (Figures 3 and 4). We observed heterogeneous hepatocytes in mice treated with linagliptin. Although lipid infiltration was present in

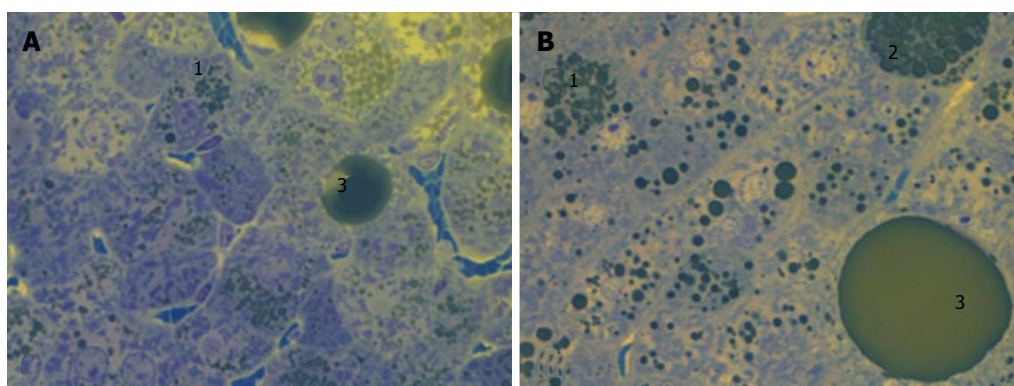




**Figure 1** Liver histology in intact *db/db* mice. A, B: Microvesicular (1) and mediovesicular (2) lipid accumulation, sporadic large lipid droplets in hepatocytes (3). Light microscopy with yellow filter of semi-thin sections stained with toluidine blue; magnification  $\times 1000$ .



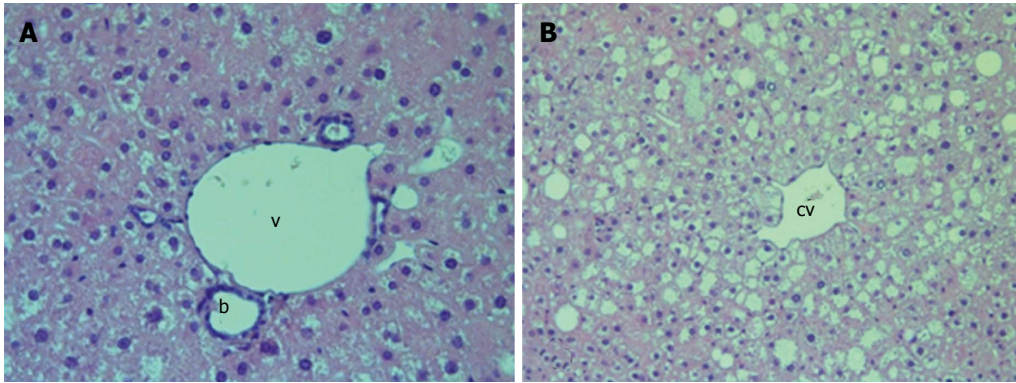
**Figure 2** Liver histology in intact *db/db* mice. A, B: The dilatation of interlobular arteries and veins (v), lymphatic vessels (L) and bile canaliculi (b) was present in most of histological preparations. H and E; magnification  $\times 400$ .



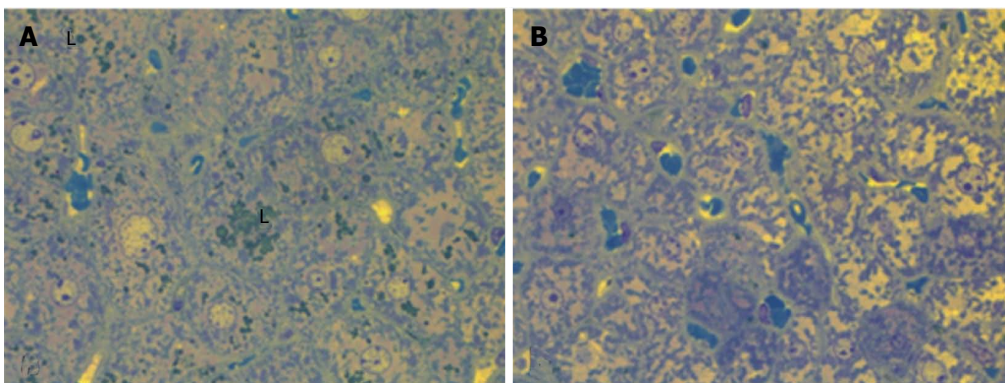
**Figure 3** Liver histology in placebo-treated *db/db* diabetic mice. A, B: Microvesicular (1) and mediovesicular (2) lipid accumulation, sporadic large lipid droplets in hepatocytes (3). Light microscopy with yellow filter of semi-thin sections stained with toluidine blue; magnification  $\times 1000$ .

some hepatocytes, other cells demonstrated preserved morphology (Figure 5). In the periportal zones, numerous diplocariocytes were found, which may be interpreted as a regenerative sign. In linagliptin-treated mice, compared to intact or placebo-treated mice, the dilation of blood and lymphatic vessels of the portal tracts, sublobular and central veins was less profound, and edema of the perisinusoidal lymphatic spaces was diminished (Figure 6). The severity of liver steatosis

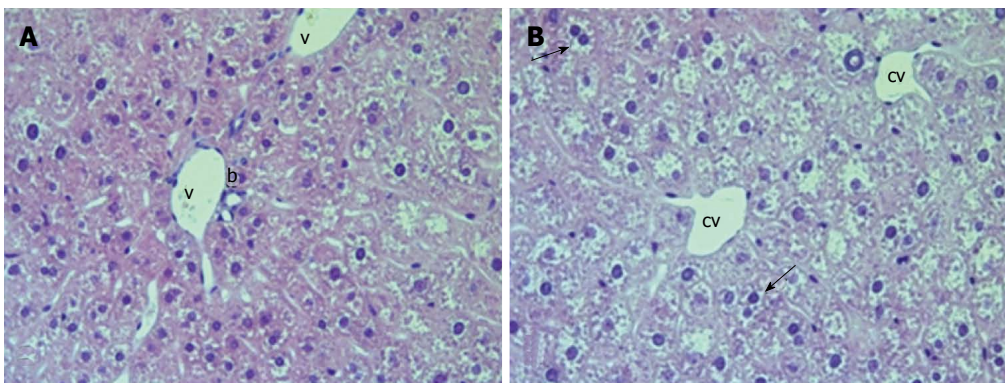
in the linagliptin group was alleviated. Specifically, the proportion of hepatocytes with a high numeral density of lipid droplets ( $> 15$  per cell) was reduced significantly in the linagliptin group compared to the placebo group ( $20.7\% \pm 3.6\%$  and  $50.4\% \pm 3.1\%$ , respectively,  $P = 0.0007$ ; Figure 7). The mean percent of hepatocytes with lipid droplets per field of view was also decreased (linagliptin:  $64.9\% \pm 5.8\%$ , placebo:  $92.4\% \pm 1.7\%$ ,  $P = 0.0002$ ), mostly due to the reduction of microvesicular



**Figure 4** Liver histology in placebo-treated *db/db* mice. A: Dilatation of the interlobular veins (v), lymphatic vessels and bile ducts (b). H and E; magnification  $\times 400$ ; B: Extension of the central vein (cv), vacuolar degeneration of hepatocytes. H and E; magnification  $\times 200$ .



**Figure 5** Liver histology in linagliptin-treated *db/db* diabetic mice. Heterogeneity of the changes of hepatocytes: A: Microvesicular lipid accumulation (L); B: No lipid accumulation. Light microscopy with yellow filter of semi-thin sections stained with toluidine blue; magnification  $\times 1000$ .



**Figure 6** The liver of linagliptin-treated *db/db* diabetic mouse. The dilatation of blood and lymphatic vessels of portal tracts, central veins was less profound. Numerous diplocariocytes were present (arrows). V: The vein of portal tract; b: Bile duct of portal tract; cv: Central vein. H and E; magnification  $\times 400$ .

and mediovesicular lipid accumulation (Figure 8).

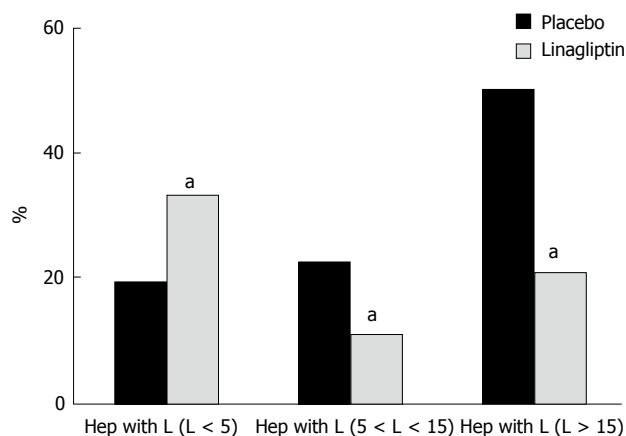
#### Ultrastructural changes in the liver

In the hepatocytes of 18-wk-old intact mice, areas of hyperplasia of the smooth endoplasmic reticulum (ER) and lipid inclusions, predominantly small ones, were found *via* electron microscopy. We observed intense exocytosis of lipids into the Disse space and interstitial areas between hepatocytes. The hyperplasia of the microvilli on the vascular poles of hepatocytes was

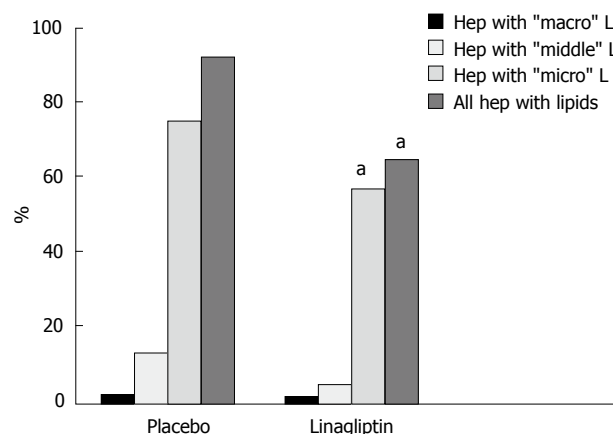
in concordance with enhanced lipid transport from the cells. The mitochondria were concentrated on the bile poles of hepatocytes and appeared condensed, with increased matrix density and indistinct cristae. Compartmentalization of the complexes of mitochondria and rough ER was found in many cells. We observed 1-2 active Golgi complexes, residual bodies and autophagosomes in addition to bile capillaries (Figure 9).

Ultrastructural changes in the placebo-treated mice were similar to those in intact animals. We observed

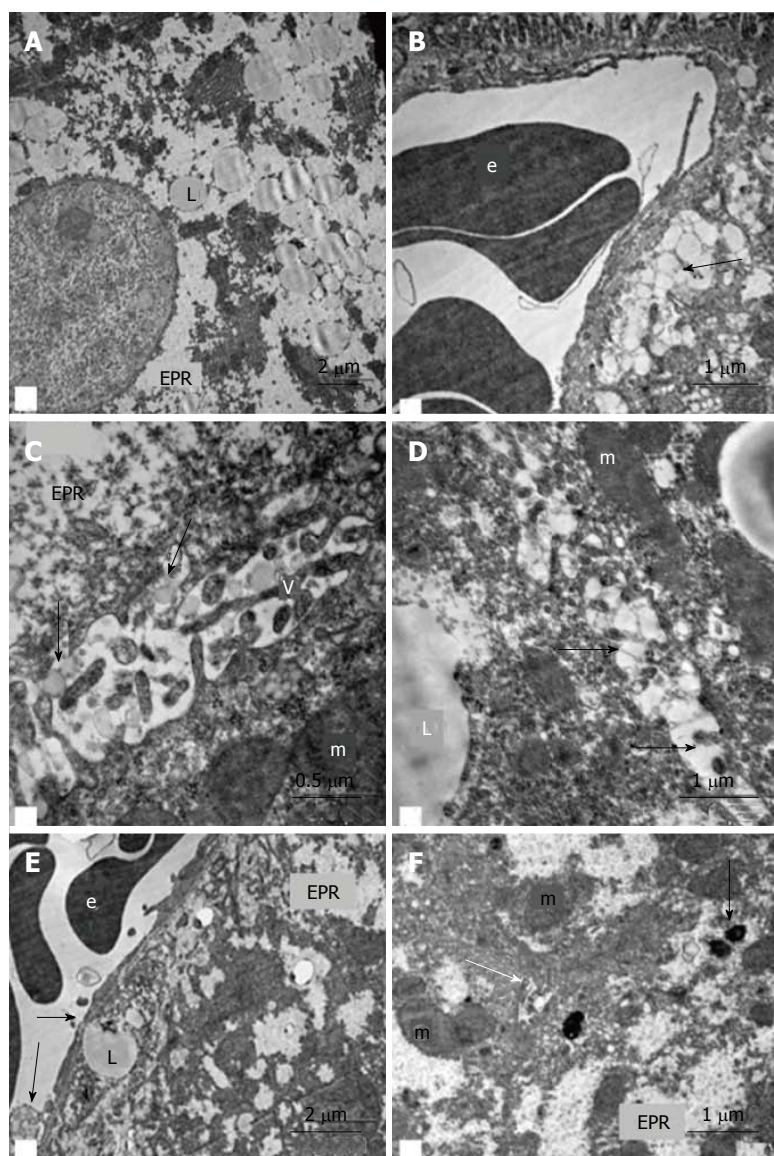




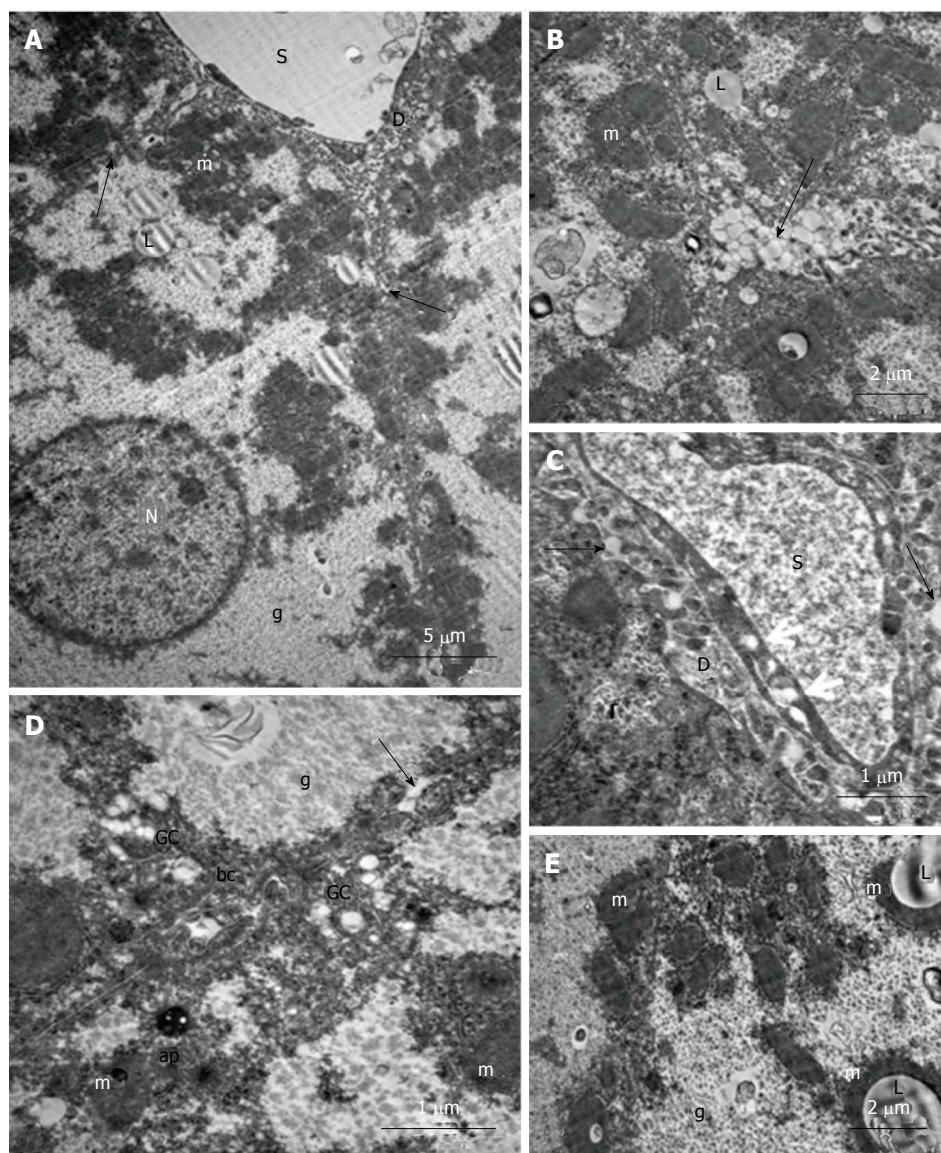
**Figure 7** The mean proportions of hepatocytes with different densities of lipid droplets in linagliptin-treated and placebo-treated *db/db* mice. The percent of hepatocytes with high density of lipid droplets (more than 15 droplets per cell) is reduced in linagliptin-treated mice compared to placebo-treated mice (hep, hepatocyte, L < 5, less than 5 lipid droplets per cell, L > 15, more than 15 lipid droplets per cell, <sup>a</sup>*P* < 0.05).



**Figure 8** The distribution of hepatocytes with lipid inclusions depending on the size of lipid droplets in linagliptin-treated and placebo-treated *db/db* mice. The reduction in the numeral density of hepatocytes with micro-sized, mediosized and macrosized droplets in linagliptin-treated mice (hep, hepatocyte, <sup>a</sup>*P* < 0.05 vs placebo group).



**Figure 9** Ultrastructural changes in the hepatocytes of intact *db/db* mice. A: Fields of "foamy" hyperplastic smooth ER and fields of glycogen, lipid inclusions in the cytoplasm of hepatocytes; B and E: Pronounced exocytosis of vacuoles with lipid content into the Disse space (arrows); C and D: Pronounced exocytosis of vacuoles with lipid content into gaps between hepatocytes (arrows); F: The bile capillary (white arrow) and compartments of the mito-ER-complexes (complexes from ER and mitochondria), active Golgi complexes, residual bodies and autophagosomes (black arrow) at the biliary poles of hepatocytes. V: Microvilli on the lateral surface of hepatocytes; L: Lipid inclusions; m: Mitochondria; e: Erythrocyte; EPR: Endoplasmic reticulum.



**Figure 10 Ultrastructural changes in the hepatocytes of placebo-treated db/db mice.** A: Fatty degeneration, numerous compartments of mito-ER-complexes, free ribosomes and polyosomes, pronounced hyperplasia of the microvilli on the vascular poles and lateral sites of parenchymal cells, enlarged Disse spaces; the arrows indicate the extension between the lateral surfaces of adjacent hepatocytes; B: Hyperplasia of microvilli on the lateral parts of the hepatocytes and transport of lipid inclusions (arrow) into spaces between hepatocytes; C: The transport of lipid inclusions into Disse spaces (arrows), transport vacuoles into the cytoplasm of endothelial sinusoidal cells; D: Active Golgi complexes, autophagosomes with dark content and ribosomes in peribiliary areas of hepatocytes; the arrow shows the transport of lipid inclusions into the gap between hepatocytes; E: Structural complexes of lipid inclusions with mitochondria. ap: Autophagosome; g: Glycogen granules; D: The Disse space; bc: Bile capillary; GC: Golgi complex; L: Lipid inclusion; m: Mitochondria; s: Lumen of the sinusoid; e: Erythrocyte; N: The nucleus.

microvesicular and mediovesicular lipid inclusions, numerous compartments of mitochondria-ER complexes, as well as marked hyperplasia of the microvilli on the vascular and lateral poles of hepatocytes. The Disse space and gaps between hepatocytes were enlarged (Figure 10A). Intense exocytosis of small lipid droplets into the gaps between hepatocytes was observed (Figure 10B). Additionally, we found the exocytosis of lipid-containing vacuoles into the enlarged Disse space (Figure 10C). In the peribiliary areas of some hepatocytes, we observed 1-3 active Golgi complexes and autophagosomes with dense content and ribosomes (Figure 10D). Mitochondria complexes with lipid inclusions were also present (Figure 10E).

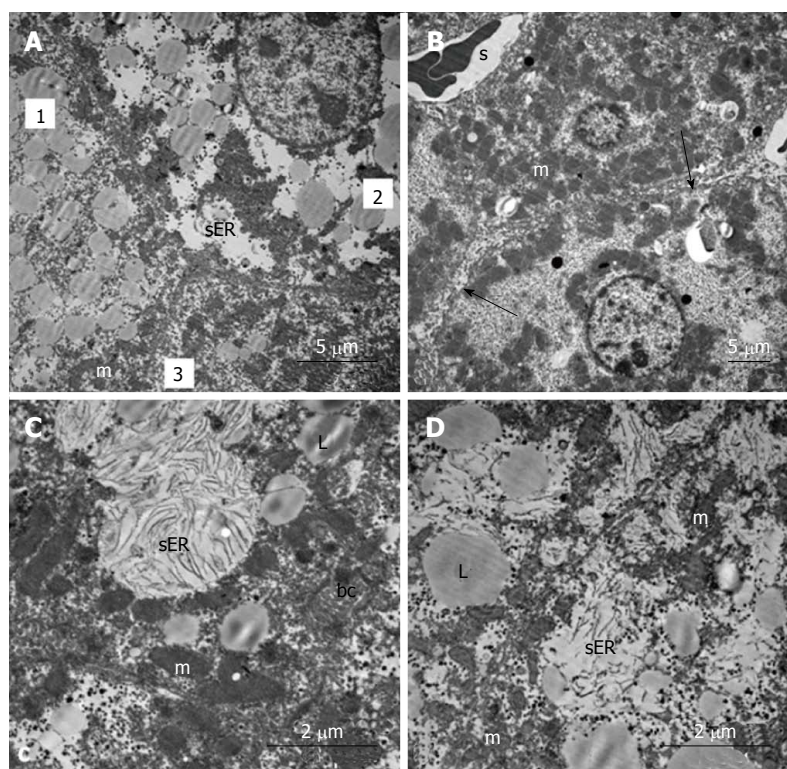
In the livers of linagliptin-treated mice, we observed heterogeneous ultrastructural changes. There were parenchymal cells with lipid accumulation and hyperplasia of the smooth ER (Figure 11A and B). Some hepatocytes demonstrated preserved (almost normal) cellular organization. In the cytoplasm of other cells, we observed zones of destructive ER membranes and quantities of

free ribosomes and polyribosomes (Figure 11C and D). Aggregates from mitochondria, rough ER and lipids were present in some images (Figure 12A). In the peribiliary zones of some hepatocytes, we found myelin structures, vacuoles of Golgi complex and autophagosomes (Figure 12B). Hepatocytes with no ER hyperplasia and a homogenous distribution of mitochondria were observed in the livers from linagliptin-treated mice. The presence of large vacuoles with lipid content in the cytoplasm of endothelial sinusoidal cells was another structural feature of this group (Figure 12D).

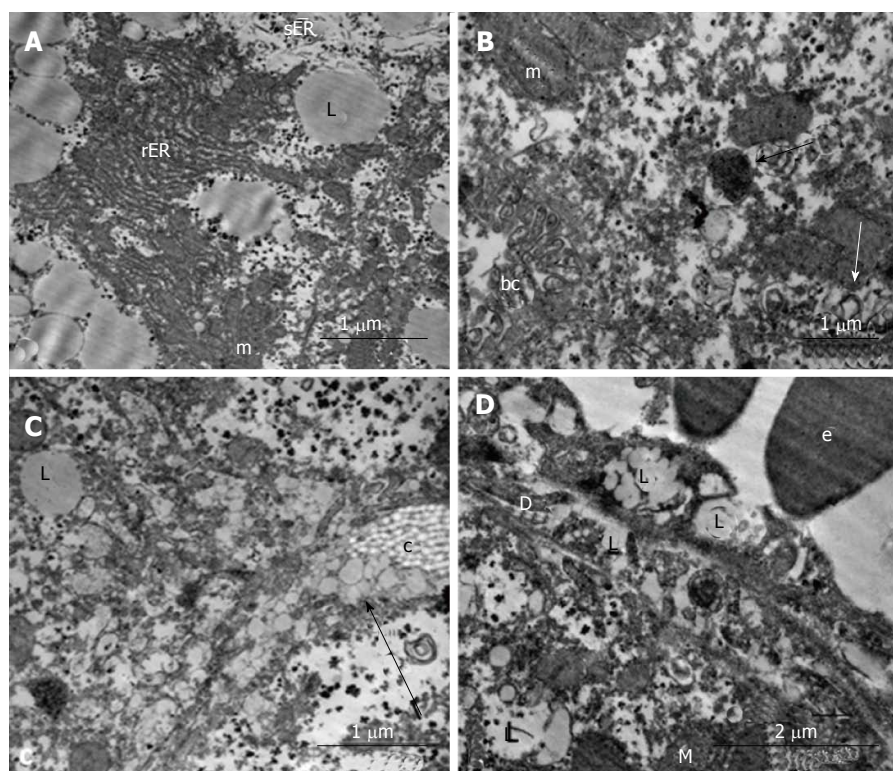
#### Staining for LYVE-1

In 18-wk-old intact or placebo-treated diabetic mice, we detected immunohistochemical staining for LYVE-1 in the endothelial cells of interlobular lymphatic vessels and on the membranes of some endothelial sinusoidal cells. The LYVE-1 staining was intensified in linagliptin-treated animals compared to intact or saline-treated animals (Figure 13). An enlarged LYVE-1 reaction area was observed in the linagliptin group as revealed by





**Figure 11 Ultrastructural changes in the hepatocytes of linagliptin-treated *db/db* mice.** A: Heterogeneity of the hepatocytes: Cells with numerous lipid inclusions (1), cells with areas of hyperplasia of smooth ER and lipid vacuoles (2), cells with a relatively uniform distribution of organelles and rare lipid inclusions (3); B: Cells without hyperplasia of the smooth ER with a relatively homogenous distribution of organelles; distinct microvilli on vascular poles of the hepatocytes and on the lateral sides of parenchymal cells; the extension of spaces between hepatocytes (arrows); C and D: Plots of clusters of smooth ER membranes. L: Lipid inclusion; m: Mitochondria; s: Lumen of the sinusoid; SER: Smooth endoplasmic reticulum; bc: Bile capillary.



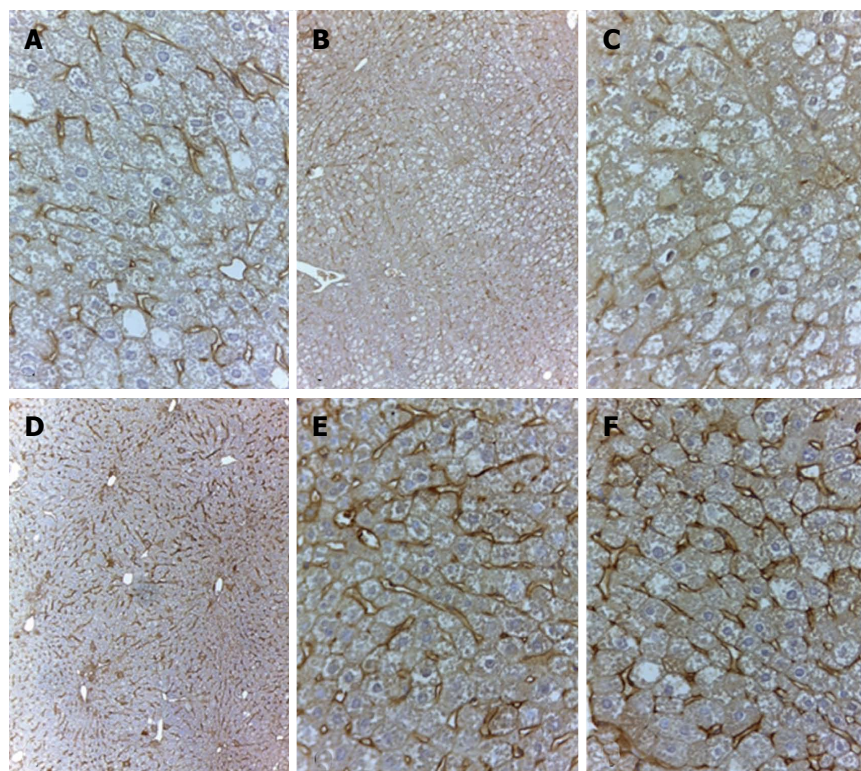
**Figure 12 Ultrastructural changes in the hepatocytes of linagliptin-treated *db/db* mice.** A: The complexes from the mitochondria, rough ER and lipid droplets; B: Mitochondria with separate granular ER profiles, myelin structures (white arrow), autophagosomes with electrondark content and ribosomes (black arrow) nearby the bile capillaries with pronounced microvilli; C: The transport of lipids into the gaps between hepatocytes (arrow); D: Large vacuoles in the cytoplasm of endothelial cells in the sinusoids. rER: Rough endoplasmic reticulum; sER: Smooth endoplasmic reticulum; bc: Bile capillary; D: The Disse space; c: A tuft of collagen; L: Lipid inclusion; m: Mitochondria; e: Erythrocyte.

morphometric analysis (Figure 14).

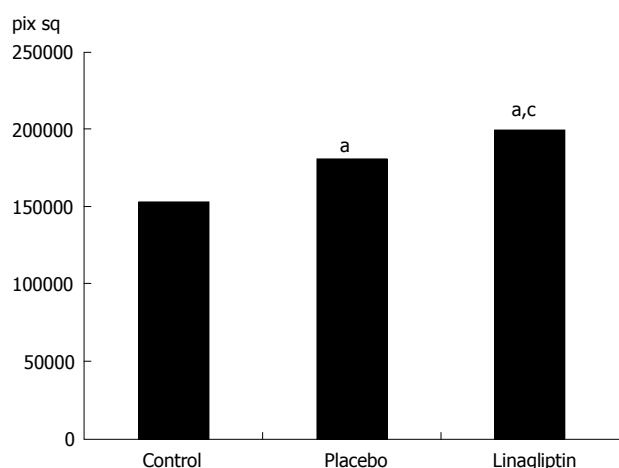
## DISCUSSION

DPP4 inhibitors are a relatively new class of hypoglycemic agents that have a broad application for the treatment of diabetes worldwide. A growing body of evidence indicates that DPP4 inhibitors could produce multiple pleiotropic

effects independent of lowering glucose levels<sup>[19,20]</sup>. In this study, we demonstrated the beneficial effects of the DPP4 inhibitor linagliptin on both parenchymal and non-parenchymal hepatic cells in T2D *db/db* mice (BKS. Cg-*Dockm*<sup>+/+</sup>*Lepr*<sup>db/J</sup>). Our results demonstrate the protective effects of linagliptin on hepatocytes, sinusoidal cells and the roots of the hepatic lymphatic system in a T2D model.



**Figure 13** Immunohistochemical staining for lymphatic vessel endothelial hyaluronan receptor-1 in the liver of intact (A and B), placebo-treated (C) and linagliptin-treated (D, E and F) *db/db* mice. Staining by anti-LYVE-1 antibodies, indirect streptavidin-biotin method; A, C, E and F:  $\times 400$ ; B and D:  $\times 100$ . LYVE-1: Lymphatic vessel endothelial hyaluronan receptor-1.



**Figure 14** The area of immunohistochemical staining for lymphatic vessel endothelial hyaluronan receptor-1 in the liver of intact, placebo-treated and linagliptin-treated *db/db* mice. <sup>a</sup> $P < 0.05$  vs control group (intact animals); <sup>c</sup> $P < 0.05$  vs placebo group. pix sq: Square pixel.

Expectedly, lipid accumulation in the liver was the principal morphological finding characterizing the development of NAFLD in *db/db* mice. Specifically, microvesicular and mediovesicular steatosis were prevalent. However, we observed no evident signs of inflammation or fibrosis.

Structural changes in the ER and mitochondria were found in hepatocytes by electron microscopy. In particular, we observed compartmentalization of the complexes of mitochondria and rough ER. Although the ER and mitochondria play distinct cellular roles, these organelles also form physical interactions with each

other at sites defined as mitochondria-associated ER membranes, which are essential for calcium, lipid and metabolite exchange. In the liver, obesity leads to a marked reorganization of mitochondria-associated ER membranes resulting in mitochondrial calcium overload, compromised mitochondrial oxidative capacity and augmented oxidative stress<sup>[21]</sup>. Mitochondrial dysfunction and ER stress or the unfolded protein response contribute to hepatocyte cell death during alterations of lipid and fatty acid metabolism<sup>[22]</sup>. An association between microvesicular steatosis and apoptosis was demonstrated recently in an NAFLD diabetic model<sup>[18]</sup>.

Consistent with the findings of another research group<sup>[6,8]</sup>, we documented the amelioration of liver steatosis in linagliptin-treated animals. The phenomenon of hepatocyte heterogeneity with the emergence of a relatively preserved cell structure was observed in the linagliptin group. Additionally, the ultrastructural changes in hepatocyte ER and mitochondria were alleviated by linagliptin treatment. Because we observed a preserving effect of the DPP4 inhibitor on ER and mitochondria structure, we anticipate improvement of hepatocyte synthetic function and energy expenditure. Modulation of mitochondrial function upon DPP4 inhibition has been recently described. In a model of Western-diet induced liver steatosis, DPP4 inhibitor MK0626 significantly reduced mitochondrial incomplete palmitate oxidation and increased the indices of pyruvate dehydrogenase activity<sup>[9]</sup>.

As far as we know, we provide here the first detailed description of the morphological changes in the hepatic interstitium of *db/db* mice. The data indicate deviations in the structure of the interlobular blood vessels, hema-



tolymphatic barrier and intrahepatic lymphatic collectors. Dilation of the roots of the lymphatic system, venous collectors and bile ducts provide morphological evidence of the impairment of lymphatic drainage and bile collection in this model of NAFLD. We also observed morphological signs of enhanced lipid transport into the interstitial tissue between hepatocytes and into the Disse space in *db/db* mice. Because hepatocyte homeostasis is intimately associated with blood microcirculation and lymphatic drainage, the changes in parenchymal cells and non-parenchymal compartments of the liver in subjects with diabetes could be mutually deteriorated.

We observed immunohistochemical staining for LYVE-1 in the endothelial cells of interlobular lymphatic vessels and on the membranes of endothelial sinusoidal cells in intact and saline-treated *db/db* mice. The LYVE-1 molecule is considered the primary immunohistochemical marker of lymphatic endothelial cells<sup>[23]</sup>. Nevertheless, LYVE-1 can be expressed by other cell types, including sinusoidal cells in the liver<sup>[24,25]</sup>. As a transmembrane receptor, LYVE-1 is involved in the transport and turnover of hyaluronan and may play a role in lymphangiogenesis<sup>[26]</sup>. The reduced expression of LYVE-1 in sinusoidal cells was reported previously in human chronic hepatitis and liver cirrhosis. A loss of fenestrae in the sinusoidal endothelium was observed in the damaged areas with low LYVE-1 expression. Interestingly, LYVE-1 attenuation in the sinusoidal endothelium is one of the manifestations of capillarization and is associated with hepatic disease progression<sup>[25]</sup>. We report here that linagliptin potently enhances the expression of LYVE-1 in the endothelial cells of interlobular lymphatic vessels and on the membranes of endothelial sinusoidal cells. Considering the previously mentioned data, we speculate that this phenomenon is associated with the activation of transendothelial transport and lymphatic drainage.

Importantly, the liver histology in linagliptin-treated mice improved significantly despite the absence of an obvious effect on hyperglycemia. Other authors also observed no significant effects of linagliptin on the glucose metabolism parameters of diabetic *db/db* mice<sup>[27]</sup>. Nevertheless, it has been documented that a protective effect of linagliptin on the kidneys could be achieved independent of the hypoglycemic action in this model of diabetes<sup>[27,28]</sup>. Although some of the effects of DPP4 inhibitors could be due to an overall improvement in the metabolic parameters, no data support improvements independent of weight loss or *via* direct effects on hepatocytes *in vitro*. In experimental and clinical diabetes, DPP4 activity in the blood serum and liver does not correlate with mean glucose or glycated hemoglobin A1c levels, which are both related to hepatic lipogenesis and liver damage<sup>[29]</sup>. The glucose-independent action of linagliptin in NAFLD could be mediated, at least partially, *via* the prolongation of the GLP-1 half-life and the extending GLP-1 effects in the liver. Multiple hepatocyte signal transduction pathways appear to be activated by GLP-1 and its analogues, and both cAMP-activated protein kinase and Akt are proposed

key players in improving hepatic steatosis<sup>[3,30]</sup>.

DPP4 itself might be an important target molecule in NAFLD. The liver expresses high levels of DPP4, and recent accumulating data suggest that DPP4 is involved in the development of various chronic liver diseases, such as NAFLD, hepatitis C virus infection, and hepatocellular carcinoma. In addition to its peptidase activity, DPP4 is associated with immune stimulation, binding to and the degradation of the extracellular matrix, resistance to anti-cancer agents, and lipid accumulation. Furthermore, DPP4 is expressed in hepatic stem cells and plays a crucial role in hepatic regeneration<sup>[29]</sup>. Normal and high fat diet fed DPP4-deficient rats exhibited reduced hepatic triglycerides, accompanied by the down-regulation of lipogenesis enzymes and the parallel up-regulation of carnitine palmitoyltransferase-1, a key enzyme in fatty acid  $\beta$ -oxidation<sup>[30]</sup>. Rats with DPP4 deficiency have improved bile secretory function in a high fat diet-induced steatosis model<sup>[7]</sup>. In patients with T2D and/or morbid obesity, circulating DPP4 activity is associated with current apoptosis and liver fibrosis<sup>[31]</sup>.

Thus, it is highly plausible that the observed improvement in liver histology following linagliptin treatment could be mediated by both the prolongation of GLP-1 effects and the inhibition of hepatic DPP4 activity *per se*.

The results demonstrate the favorable effect of long-term linagliptin treatment on the liver structure of obese *db/db* mice with T2D. In this model of NAFLD, linagliptin alleviates structural signs of steatosis, and disturbances in microcirculation and lymphatic drainage. The improvement in the structural parameters of the liver in linagliptin-treated mice was independent to changes in the plasma glucose levels.

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## COMMENTS

### Background

Dipeptidyl peptidase 4 (DPP4) inhibitors are a relatively new class of hypoglycemic agents with multiple pleiotropic effects. The ability of DPP4 inhibitors to modify the development of diabetic complications remains unclear. It was recently demonstrated that some DPP4 inhibitors result in hepatic fat reduction in experimental models of non-alcoholic fatty liver disease (NAFLD). Preliminary data indicate that DPP4 inhibitors might have a beneficial effect on hepatic steatosis and serum transaminase activity, but the data on their effects on liver histology are limited.

### Research frontiers

Despite the accumulating data on the favorable influence of DPP4 inhibitors on liver steatosis, the effects of these agents on non-parenchymal cells, bile transport, microcirculation and lymphatic draining in the liver remain unknown.

### Innovations and breakthroughs

In this study, the authors demonstrated for the first time that the DPP4 inhibitor linagliptin not only alleviates liver steatosis but also diminishes structural

changes in hepatic non-parenchymal compartments in *db/db* diabetic mice. Incremental changes in the lymphatic vessel endothelial hyaluronan receptor-1 expression in the endothelial cells of interlobular lymphatic vessels and on the membranes of some endothelial sinusoidal cells under linagliptin treatment may improve impaired lymphatic drainage and sinusoid function in NAFLD. The mechanism of the beneficial effect of linagliptin seems to be glucose-independent as no obvious hypoglycemic effect of the agent was observed in this model.

## Applications

The results of this study provide further evidence that linagliptin could be a promising agent for the treatment of NAFLD in subjects with type 2 diabetes. Further studies regarding the effects of DPP4 inhibitors on liver structure and function in diabetes are urgently needed.

## Terminology

Sinusoidal cells, a non-parenchymal cell population in the liver that includes sinusoidal endothelial cells, Kupffer cells, Ito cells and Pit cells. Lymphatic vessel endothelial hyaluronan receptor-1, a transmembrane receptor for the extracellular matrix glycosaminoglycan hyaluronan.

## Peer-review

The investigation by Michurina *et al* aimed to study the effects of Linagliptin on the structural signs of non-alcoholic fatty liver disease in *db/db* mice. This is an interesting work from a basic science point of view, that may have clinical practice consequences.

## REFERENCES

- Ahmadieh H, Azar ST. Liver disease and diabetes: association, pathophysiology, and management. *Diabetes Res Clin Pract* 2014; **104**: 53-62 [PMID: 24485856 DOI: 10.1016/j.diabres.2014.01.003]
- Blaslov K, Bulum T, Zibar K, Duvnjak L. Incretin based therapies: a novel treatment approach for non-alcoholic fatty liver disease. *World J Gastroenterol* 2014; **20**: 7356-7365 [PMID: 24966606 DOI: 10.3748/wjg.v20.i23.7356]
- Samson SL, Bajaj M. Potential of incretin-based therapies for non-alcoholic fatty liver disease. *J Diabetes Complications* 2013; **27**: 401-406 [PMID: 23352496 DOI: 10.1016/j.jdiacomp.2012.12.005]
- Nakouti T, Karagiannis AK, Tziomalos K, Cholongitas E. Incretin-Based Antidiabetic Agents for the Management of Non-Alcoholic Fatty Liver Disease. *Curr Vasc Pharmacol* 2015; **13**: 649-657 [PMID: 25412688 DOI: 10.2174/157016112666141121112612]
- Carbone LJ, Angus PW, Yeomans ND. Incretin-based therapies for the treatment of non-alcoholic fatty liver disease: A systematic review and meta-analysis. *J Gastroenterol Hepatol* 2016; **31**: 23-31 [PMID: 26111358 DOI: 10.1111/jgh.13026]
- Kern M, Klötting N, Niessen HG, Thomas L, Stiller D, Mark M, Klein T, Blüher M. Linagliptin improves insulin sensitivity and hepatic steatosis in diet-induced obesity. *PLoS One* 2012; **7**: e38744 [PMID: 22761701 DOI: 10.1371/journal.pone.0038744]
- Ben-Shlomo S, Zvibel I, Rabinowich L, Goldiner I, Shlomai A, Santo EM, Halpern Z, Oren R, Fishman S. Dipeptidyl peptidase 4-deficient rats have improved bile secretory function in high fat diet-induced steatosis. *Dig Dis Sci* 2013; **58**: 172-178 [PMID: 22918684 DOI: 10.1007/s10620-012-2353-7]
- Klein T, Fujii M, Sandel J, Shibasaki Y, Wakamatsu K, Mark M, Yoneyama H. Linagliptin alleviates hepatic steatosis and inflammation in a mouse model of non-alcoholic steatohepatitis. *Med Mol Morphol* 2014; **47**: 137-149 [PMID: 24048504 DOI: 10.1007/s00795-013-0053-9]
- Aroor AR, Habibi J, Ford DA, Nistala R, Lastra G, Manrique C, Dunham MM, Ford KD, Thyfault JP, Parks EJ, Sowers JR, Rector RS. Dipeptidyl peptidase-4 inhibition ameliorates Western diet-induced hepatic steatosis and insulin resistance through hepatic lipid remodeling and modulation of hepatic mitochondrial function. *Diabetes* 2015; **64**: 1988-2001 [PMID: 25605806 DOI: 10.2337/db14-0804]
- Kanazawa I, Tanaka K, Sugimoto T. DPP-4 inhibitors improve liver dysfunction in type 2 diabetes mellitus. *Med Sci Monit* 2014; **20**: 1662-1667 [PMID: 25228119 DOI: 10.12659/MSM.890989]
- Macauley M, Hollingsworth KG, Smith FE, Thelwall PE, Al-Mrabeh A, Schweizer A, Foley JE, Taylor R. Effect of vildagliptin on hepatic steatosis. *J Clin Endocrinol Metab* 2015; **100**: 1578-1585 [PMID: 25664602 DOI: 10.1210/jc.2014-3794]
- Mashitani T, Noguchi R, Okura Y, Namisaki T, Mito A, Ishii H, Nakatani T, Kikuchi E, Moriyasu H, Matsumoto M, Sato S, An T, Morita H, Aizawa S, Tokuoka Y, Ishikawa M, Matsumura Y, Ohira H, Kogure A, Noguchi K, Yoshiji H. Efficacy of alogliptin in preventing non-alcoholic fatty liver disease progression in patients with type 2 diabetes. *Biomed Rep* 2016; **4**: 183-187 [PMID: 26893835 DOI: 10.3892/br.2016.569]
- Guedes EP, Hohl A, de Melo TG, Lauand F. Linagliptin: pharmacology, efficacy and safety in type 2 diabetes treatment. *Diabetol Metab Syndr* 2013; **5**: 25 [PMID: 23697612 DOI: 10.1186/1758-5996-5-25]
- Blech S, Ludwig-Schwellinger E, Gräfe-Mody EU, Withopf B, Wagner K. The metabolism and disposition of the oral dipeptidyl peptidase-4 inhibitor, linagliptin, in humans. *Drug Metab Dispos* 2010; **38**: 667-678 [PMID: 20086031 DOI: 10.1124/dmd.109.031476]
- Fuchs H, Runge F, Held HD. Excretion of the dipeptidyl peptidase-4 inhibitor linagliptin in rats is primarily by biliary excretion and P-gp-mediated efflux. *Eur J Pharm Sci* 2012; **45**: 533-538 [PMID: 22198311 DOI: 10.1016/j.ejps.2011.11.018]
- McCuskey RS, Ito Y, Robertson GR, McCuskey MK, Perry M, Farrell GC. Hepatic microvascular dysfunction during evolution of dietary steatohepatitis in mice. *Hepatology* 2004; **40**: 386-393 [PMID: 15368443 DOI: 10.1002/hep.20302]
- Farrell GC, Teoh NC, McCuskey RS. Hepatic microcirculation in fatty liver disease. *Anat Rec (Hoboken)* 2008; **291**: 684-692 [PMID: 18484615 DOI: 10.1002/ar.20715]
- Trak-Smayra V, Paradis V, Massart J, Nasser S, Jebara V, Fromenty B. Pathology of the liver in obese and diabetic ob/ob and db/db mice fed a standard or high-calorie diet. *Int J Exp Pathol* 2011; **92**: 413-421 [PMID: 22118645 DOI: 10.1111/j.1365-2613.2011.00793.x]
- Avogaro A, Fadini GP. The effects of dipeptidyl peptidase-4 inhibition on microvascular diabetes complications. *Diabetes Care* 2014; **37**: 2884-2894 [PMID: 25249673 DOI: 10.2337/dc14-0865]
- Korbut AI, Klimontov VV. Incretin-based therapy: renal effects. *Diabetes Mellitus* 2016; **19**: 53-63 [DOI: 10.14341/DM7727]
- Arruda AP, Pers BM, Parlakgöl G, Güney E, Inouye K, Hotamisligil GS. Chronic enrichment of hepatic endoplasmic reticulum-mitochondria contact leads to mitochondrial dysfunction in obesity. *Nat Med* 2014; **20**: 1427-1435 [PMID: 25419710]
- Malhi H, Guicciardi ME, Gores GJ. Hepatocyte death: a clear and present danger. *Physiol Rev* 2010; **90**: 1165-1194 [PMID: 20664081 DOI: 10.1152/physrev.00061.2009]
- Akishima Y, Ito K, Zhang L, Ishikawa Y, Orikasa H, Kiguchi H, Akasaka Y, Komiyama K, Ishii T. Immunohistochemical detection of human small lymphatic vessels under normal and pathological conditions using the LYVE-1 antibody. *Virchows Arch* 2004; **444**: 153-157 [PMID: 14722766 DOI: 10.1007/s00428-003-0950-8]
- Mouta Carreira C, Nasser SM, di Tomaso E, Padera TP, Boucher Y, Tomarev SI, Jain RK. LYVE-1 is not restricted to the lymph vessels: expression in normal liver blood sinusoids and down-regulation in human liver cancer and cirrhosis. *Cancer Res* 2001; **61**: 8079-8084 [PMID: 11719431]
- Arimoto J, Ikura Y, Suekane T, Nakagawa M, Kitabayashi C, Iwasa Y, Sugioka K, Naruko T, Arakawa T, Ueda M. Expression of LYVE-1 in sinusoidal endothelium is reduced in chronically inflamed human livers. *J Gastroenterol* 2010; **45**: 317-325 [PMID: 19908110 DOI: 10.1007/s00535-009-0152-5]
- Yu M, Zhang H, Liu Y, He Y, Yang C, Du Y, Wu M, Zhang G, Gao F. The cooperative role of SIP3 with LYVE-1 in LMW-HA-induced lymphangiogenesis. *Exp Cell Res* 2015; **336**: 150-157 [PMID: 26116468 DOI: 10.1016/j.yexcr.2015.06.014]
- Sharkovska Y, Reichetzer C, Alter M, Tsuprykov O, Bachmann S, Secher T, Klein T, Hoher B. Blood pressure and glucose independent



- renoprotective effects of dipeptidyl peptidase-4 inhibition in a mouse model of type-2 diabetic nephropathy. *J Hypertens* 2014; **32**: 2211-2223; discussion 2223 [PMID: 25215436 DOI: 10.1097/HJH.0000000000000328]
- 28 **Klimontov VV**, Bgatova NP, Gavrilova JuS, Ischenko IJu, Myakina NE, Michurina SV, Zavjalov EL. Linagliptin allieviate renal injury in a model of type 2 diabetic nephropathy. *Diabetes* 2015; **64** (S 1): A144 [DOI: 10.2337/db16-382-651]
  - 29 **Itou M**, Kawaguchi T, Taniguchi E, Sata M. Dipeptidyl peptidase-4: a key player in chronic liver disease. *World J Gastroenterol* 2013; **19**: 2298-2306 [PMID: 23613622 DOI: 10.3748/wjg.v19.i15.2298]
  - 30 **Ben-Shlomo S**, Zvibel I, Shnell M, Shlomain A, Chepurko E, Halpern Z, Barzilai N, Oren R, Fishman S. Glucagon-like peptide-1 reduces hepatic lipogenesis via activation of AMP-activated protein kinase. *J Hepatol* 2011; **54**: 1214-1223 [PMID: 21145820 DOI: 10.1016/j.jhep.2010.09.032]
  - 31 **Williams KH**, Vieira De Ribeiro AJ, Prakoso E, Veillard AS, Shackel NA, Brooks B, Bu Y, Cavanagh E, Raleigh J, McLennan SV, McCaughan GW, Keane FM, Zekry A, Gorrell MD, Twigg SM. Circulating dipeptidyl peptidase-4 activity correlates with measures of hepatocyte apoptosis and fibrosis in non-alcoholic fatty liver disease in type 2 diabetes mellitus and obesity: A dual cohort cross-sectional study. *J Diabetes* 2015; **7**: 809-819 [PMID: 25350950 DOI: 10.1111/1753-0407.12237]

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Observational Study

# Effect of pioglitazone on nerve conduction velocity of the median nerve in the carpal tunnel in type 2 diabetes patients

Sudip Chatterjee, Debmalya Sanyal, Sourav Das Choudhury, Mili Bandyopadhyay, Suraj Chakraborty, Arabinda Mukherjee

Sudip Chatterjee, Mili Bandyopadhyay, Department of Medicine, Vivekananda Institute of Medical Sciences, Ramakrishna Mission Seva Pratishthan, Kolkata 700026, West Bengal, India

Debmalya Sanyal, Department of Endocrinology, KPC Medical College, Ramakrishna Mission Seva Pratishthan, Kolkata 700026, West Bengal, India

Sourav Das Choudhury, Department of Medicine, Gitaram Hospital, Berhampore, Murshidabad 742187, West Bengal, India

Suraj Chakraborty, Arabinda Mukherjee, Department of Neurology, Vivekananda Institute of Medical Sciences, Ramakrishna Mission Seva Pratishthan, Kolkata 700026, West Bengal, India

**Author contributions:** Chatterjee S, Sanyal D, Das Choudhury S and Mukherjee A contributed to study conception and design; Chatterjee S, Sanyal D, Bandyopadhyay M and Chakraborty S contributed to data acquisition; Chatterjee S, Sanyal D and Das Choudhury S contributed to data analysis and interpretation, and writing of article; all authors contributed to editing, reviewing and final approval of article.

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**Correspondence to:** Dr. Debmalya Sanyal, MBBS, MD, DM, MRCP, Associate Professor, Consultant Endocrinologist, Department of Endocrinology, KPC Medical College, Ramakrishna Mission Seva Pratishthan, 99, Sarat Bose Road, Kolkata 700026, West Bengal, India. [dr\\_debmalya@hotmail.com](mailto:dr_debmalya@hotmail.com)  
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## Abstract

### AIM

To evaluate the impact of pioglitazone pharmacotherapy in median nerve electrophysiology in the carpal tunnel among type 2 diabetes patients.

### METHODS

The study was executed in patients with type 2 diabetes, treated with oral drugs, categorized under pioglitazone or non-pioglitazone group (14 in each group), and who received electrophysiological evaluation by nerve conduction velocity at baseline and 3 mo.

## RESULTS

At 3 mo, pioglitazone-category had inferior amplitude in sensory median nerve [8.5 interquartile range (IQR) = 6.5 to 11.5] *vs* non-pioglitazone 14.5 (IQR 10.5 to 18.75)] ( $P = 0.002$ ). Non-pioglitazone category displayed amelioration in amplitude in the sensory median nerve [baseline 13 (IQR = 9 to 16.25) *vs* 3 mo 8.5 (IQR = 6.5 to 11.5)] ( $P = 0.01$ ) and amplitude in motor median nerve [baseline 9 (IQR = 4.75 to 11) *vs* 3 mo 6.75 (IQR = 4.75 to 10.25)] ( $P = 0.049$ ); and deterioration of terminal latency of in motor ulnar nerve [baseline 2.07 (IQR = 1.92 to 2.25) *vs* 3 mo 2.16 (IQR = 1.97 to 2.325)] ( $P = 0.043$ ). There was amelioration of terminal latency in sensory ulnar nerve [baseline 2.45 (IQR = 2.315 to 2.88) *vs* 3 mo 2.37 (IQR = 2.275 to 2.445) for pioglitazone group ( $P = 0.038$ ).

## CONCLUSION

Treatment with pioglitazone accentuates probability of compressive neuropathy. In spite of comparable glycemic control over 3 mo, patients treated with pioglitazone showed superior electrophysiological parameters for the ulnar nerve. Pioglitazone has favourable outcome in nerve electrophysiology which was repealed when the nerve was subjected to compressive neuropathy.

**Key words:** Pioglitazone; Adipocytes; Diabetes mellitus; Neuropathy; Carpal tunnel

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**Core tip:** Significant findings of the study: (1) Non-pioglitazone group showed favourable outcome in amplitude in the sensory and motor median nerve, and aggravation of terminal latency of motor ulnar nerve; and (2) Pioglitazone group showed favourable outcome of terminal latency in sensory ulnar nerve. What this study adds: (1) Pioglitazone has beneficial effect on nerve electrophysiology; and (2) The beneficial effect is nullified by the higher risk of compressive neuropathy conferred.

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## INTRODUCTION

The carpal tunnel is a fibro-osseous space in the wrist, bound anteriorly by the transverse carpal ligament and posteriorly by the pisiform and tubercle of scaphoid in the proximal part; and the tubercle of trapezoid and hook of hamate in the distal part<sup>[1]</sup>. Nine digital flexor tendons and the motor and sensory divisions of the median nerve pass through it, which also contains small amounts of

adipose tissue. Pioglitazone, a peroxisome proliferator activator receptor gamma (PPAR- $\gamma$ ) agonist, is an oral antidiabetic agent. In recent years, however, the use of pioglitazone is somewhat decreasing in patients with type 2 diabetes due to its adverse effects including edema, heart failure, bone fractures and the possible risk for bladder cancer. Animal studies have demonstrated the conversion of pre-adipocytes to adipocytes under the influence of pioglitazone, although the mechanisms continue to remain elusive<sup>[2,3]</sup>. In confined spaces like the orbit, this action has been known to cause compressive symptoms in a subgroup of patients. The incidence is higher when there is associated thyroid disease<sup>[4]</sup>. The algorithms available for clinical and electro-diagnostic evaluation of carpal tunnel syndrome (CTS) continue to evolve. After assessment by standard tests (*viz.* "distal median motor latency", "antidromic sensory recording from median nerve"), CTS can be diagnosed and classified by severity from "extreme" to "mild"<sup>[5]</sup>. For the "distal median motor latency" test, "onset motor latency > 4.2 milliseconds is abnormal", so also is a "compound muscle action potential (CMAP) amplitude < 5 mV"<sup>[6]</sup>. Extreme CTS cases are further evaluated by motor comparison study. In positive cases, needle electromyography is imperative<sup>[5]</sup>.

Accordingly, CTS can be electro-diagnostically grouped into 5 grades, as follows: "Grade 1 - Very mild CTS - normal standard tests and abnormal comparative tests; Grade 2 - Mild CTS - abnormal sensory with a normal motor response; Grade 3 - Moderate CTS - abnormal median sensory and motor response; Grade 4 - Severe CTS -absence of sensory response and abnormal distal motor latency; Grade 5 - Extreme CTS - absence of median motor and sensory responses"<sup>[7,8]</sup>.

We felt that as the carpal tunnel was a closed space with the presence of fatty tissue, it was possible that treatment with pioglitazone could decelerate nerve conduction of the median nerve. In order to generate this hypothesis we measured "terminal latency" and "amplitude" of the motor and sensory divisions of the median nerve over a fixed distance spanning the wrist and covering the carpal tunnel in patients of type 2 diabetes. This was done at baseline and after 3 mo in two matched groups of type 2 diabetes patients, one on treatment with pioglitazone and the other without.

## MATERIALS AND METHODS

A single centre, prospective, comparative case-series was studied between June 2012 and September 2012 at a tertiary care institute in Kolkata. The study subjects comprised of patients with type 2 diabetes mellitus aged between 18 and 65 years attending the diabetic clinic, treated with oral anti-diabetic agents and complying to undergo electrophysiological testing by nerve conduction velocity (NCV) study at two time points, once at the baseline and later at a gap of 3 mo. Female patients were eligible to participate if they were non-pregnant

and willing to adopt standard contraceptive methods over the next 6 mo. The exclusion criteria were clinical evidence of neuropathy or nephropathy, poor control of diabetes as defined by a glycated hemoglobin (HbA<sub>1c</sub>) over 9% (75 mmol/mol); current treatment with insulin or likelihood of insulin treatment over the next 6 mo; electrophysiologically evident CTS, contraindication to pioglitazone use; myocardial infarction in the last 6 mo; and presence of other causes of CTS like rheumatoid arthritis, untreated hypothyroidism and pregnancy. For the median nerve, distal motor latency of Abductor pollicis brevis was measured by stimulating 3 cm above distal wrist crease. For the ulnar nerve Distal motor latency of Abductor digiti minimi was measured by stimulating 3 cm above distal wrist crease with elbow flexed at 90°. NCV evaluation was performed at baseline and 3 mo. The authors feel that NCV evaluation at 3 mo increases the sensitivity of diagnosing early and asymptomatic CTS.

The electro-diagnostic criteria for CTS used in our study were as follows: (1) Distal median motor latency > 4.2 ms; (2) Difference between distal motor latency of median and ulnar nerve > 1.1 ms; (3) Difference between distal sensory latency of median nerve and ulnar nerve > 0.2 ms; (4) Difference between median and ulnar sensory latency on stimulating fourth digit and recording from wrist at equal distance > 0.2 ms; (5) Difference between median and ulnar sensory latency on stimulating thumb and recording from wrist at equal distance > 0.4 ms; and (6) Palm wrist conduction: Difference between median and ulnar sensory latency across 8 cm > 0.4 ms.

After a run in period of 1 mo, the HbA<sub>1c</sub> was reassessed. Those with HbA<sub>1c</sub> over 7.5% (58 mmol/mol) were excluded from further study. The patients had their diabetes controlled on oral agents and belonged to either pioglitazone (Group 1) or non-pioglitazone group (Group 2) depending on whether they were receiving the drug as a part of their current therapy. Patients with electrophysiological evidence of CTS on NCV were excluded from further study ( $n = 34$ ) and were labeled as Group 3. The remaining patients, 14 each in Groups 1 and 2, were requested to continue their usual diabetes treatment and were seen in the clinic every 6 wk, when fasting and 2 h post prandial blood sugar (FBS and PPBS) were checked and a clinical evaluation performed. At the end of 3 mo, HbA<sub>1c</sub> level was re-estimated. The NCV study was repeated at the end of 3 mo. All the electrophysiology studies were done by the same observer who was not aware of the treatment status, and the parameters studied were terminal latency and amplitude in the motor component of left median nerve between the elbow and the wrist (L-M-motor-ew-TL and L-M-motor-ew-Amp), and also the sensory component of the same (L-M-sensory-TL and L-M-sensory-Amp); the terminal latency and amplitude in the motor component of left ulnar nerve across the wrist (L-U-motor-aw-TL and L-U-motor-aw-Amp), and also the sensory component of the same (L-U-sensory-TL and L-U-sensory-Amp).

Data have been summarized by routine descriptive statistics, and key proportions expressed with their 95%CI. Since the number of patients in each group was 14, non-parametric tests have been used for both inter-group and intra-group comparisons of all parameters studied. Numerical variables were compared between groups by Mann-Whitney *U* test. Categorical variables were compared between groups by Fisher's exact test.  $\chi^2$  test for trend analysis was used where applicable. Median values [with interquartile range (IQR)] of age, all parameters of electrophysiological assessment in NCV and HbA<sub>1c</sub> over time were analyzed for statistically significant change by Wilcoxon matched pairs signed rank sum test. Median FBS and PPBS values over time were assessed for statistically significant change by Friedman's analysis of variance (ANOVA) with "Dunn's multiple comparison test" as post hoc test. All analyses were two-tailed and  $P < 0.05$  was considered statistically significant. Statistical Version 6 (Tulsa, Oklahoma: StatSoft Inc., 2001) and GraphPad Prism version 4 (San Diego, California: GraphPad Software Inc., 2005) software were used for analysis. The statistical review of the study was performed by a biomedical statistician.

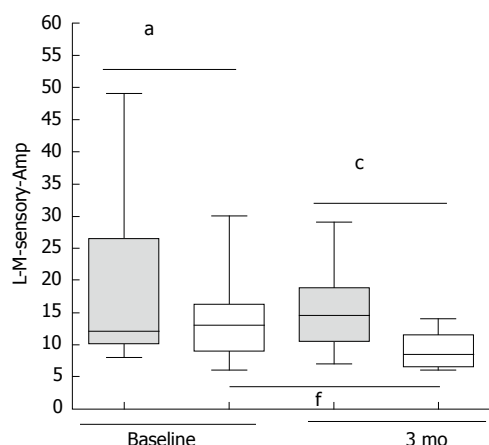
## RESULTS

Data of all the 28 patients without electrophysiological evidence of CTS on NCV were analyzed. As illustrated in Table 1, demography, duration of diabetes and baseline characteristics was comparable in the two groups<sup>[9]</sup>.

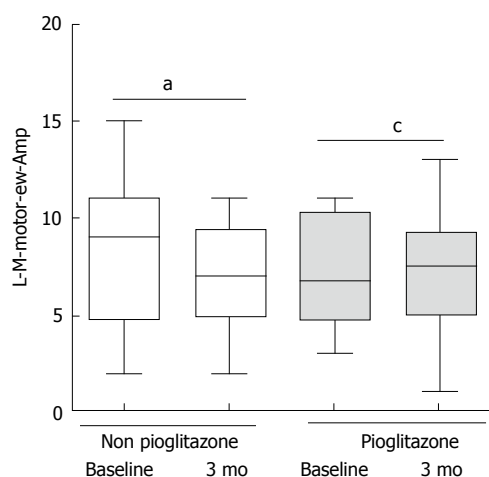
At the end of 3 mo, Group 1 patients had higher median amplitude in the sensory component of left median nerve [Group 2 8.5 (IQR = 6.5 to 11.5) vs Group 1 14.5 (IQR 10.5 to 18.75)] ( $P = 0.002$ ) (Figure 1). There was improvement in median amplitude in the sensory component of left median nerve [Baseline 13 (IQR = 9 to 16.25) vs 3 mo 8.5 (IQR = 6.5 to 11.5)] for Group 2 patients) (Figure 1). In the same group, there was improvement in median amplitude in the motor component of left median nerve [baseline 9 (IQR = 4.75 to 11) vs 3 mo 6.75 (IQR = 4.75 to 10.25)] ( $P = 0.049$ ) (Figure 2). Higher amplitude indicated greater delay in nerve conduction<sup>[9]</sup>.

The HbA<sub>1c</sub> values at the end of 3 mo were comparable between groups ( $P = 0.809$ ), but the pioglitazone group showed improvement {baseline value: 7.1% (54 mmol/mol) [IQR = 6.2% (44 mmol/mol) - 7.8 % (62 mmol/mol)] to 3 mo value: 6.3% (45 mmol/mol) [IQR = 6% (42 mmol/mol) - 6.8% (51 mmol/mol)]} ( $P = 0.002$ ). The FBS and PPBS values were comparable between Groups 1 and 2 at all time-points (data on file, not shown). There was worsening of median terminal latency of the motor component of left ulnar nerve [baseline 2.07 (IQR = 1.92 to 2.25) vs 3 mo 2.16 (IQR = 1.97 to 2.325) for Non pioglitazone group] ( $P = 0.043$ ) (Figure 3). There was improvement of median terminal latency in the sensory component of left ulnar nerve [baseline 2.45 (IQR = 2.315 to 2.88) vs 3 mo 2.37 (IQR = 2.275 to 2.445) for pioglitazone





**Figure 1** Amplitude in sensory component of Left Median nerve at baseline and 3 mo (Shaded bar: Pioglitazone arm; White bar: Non pioglitazone arm). <sup>a</sup> $P = 0.496$ , <sup>c</sup> $P = 0.002$  (Mann-Whitney *U* Test); <sup>f</sup> $P = 0.01$  (Wilcoxon matched pairs signed rank sum test). L-M-sensory-Amp: Amplitude in sensory component of Left Median nerve.

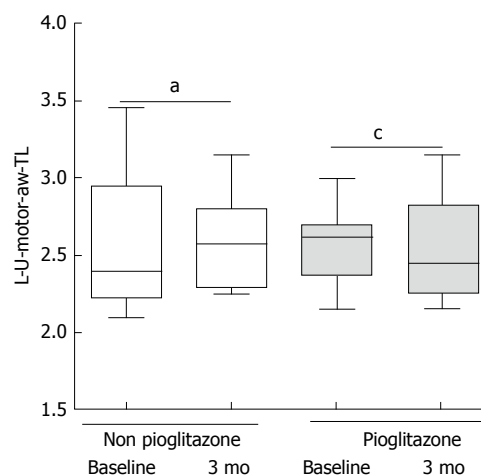


**Figure 2** Amplitude in motor component of Left Median nerve in the segment between elbow and wrist at baseline and 3 mo (Shaded bar: Pioglitazone; White bar: Non pioglitazone arm). <sup>a</sup> $P = 0.049$ , <sup>c</sup> $P = 0.964$  (Wilcoxon matched pairs signed rank sum test). L-M-motor-ew-Amp: Amplitude in motor component of Left Median nerve in the segment between elbow and wrist.

group] ( $P = 0.038$ ) (Figure 4). Higher terminal latency indicates greater delay in nerve conduction. None of the patients developed symptoms of CTS at the end of 3 mo<sup>[9]</sup>.

## DISCUSSION

Pioglitazone is widely used in the pharmacotherapy of type 2 diabetes mellitus. Luciferase reporter assay has confirmed that pioglitazone stimulates preadipocyte multiplication by augmenting S and G(2)/M cell-cycle entry by amplifying the effect of PPAR $\gamma$  on cyclin-dependent kinase inhibitors by engaging 3T3-L1 preadipocytes, especially with p16(Ink4a) (p16) centered<sup>[2]</sup>. Preclinical studies show that pioglitazone produces an increase in subcutaneous adipocyte surface and whole body adiposity<sup>[10,11]</sup>. Although mature visceral adipocytes have



**Figure 3** Terminal Latency in motor component of Left Ulnar nerve across wrist at baseline and 3 mo (Shaded bar: Pioglitazone; White bar: Non pioglitazone arm). <sup>a</sup> $P = 0.043$ , <sup>c</sup> $P = 0.055$  (Wilcoxon matched pairs signed rank sum test). L-U-motor-aw-TL: Terminal Latency in motor component of Left Ulnar nerve across wrist.

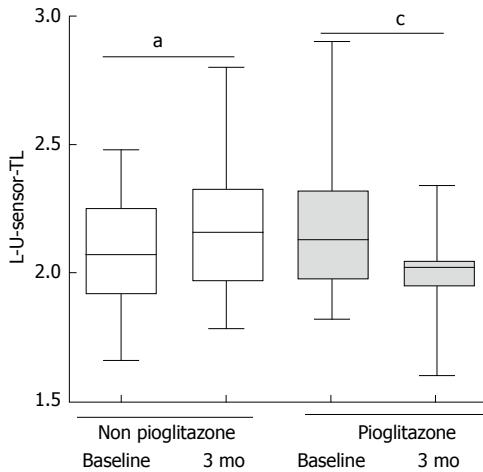
**Table 1** Baseline demographic and clinical summary of the study subjects

	Pioglitazone ( <i>n</i> = 14)	Non-pioglitazone ( <i>n</i> = 14)	<i>P</i> value
Gender (male:female)	7 (50%):7 (50%)	6 (42.86%):8 (57.14%)	1
Age (yr)	42 (35.5-52.5)	46 (42.75-51.75)	0.333
Diabetes duration (yr)	2 (1-5)	5.5 (2.75-10.25)	0.072
L-M-motor-ew-TL	3.5 (3-4)	3 (3-4)	0.756
L-M-motor-ew-Amp	6.5 (4.75-10.25)	9 (4.75-11)	0.431
L-M-sensory-TL	2 (2-3)	3 (2-3)	0.575
L-M-sensory-Amp	12 (9.75-26.5)	13 (9-16.5)	0.496
L-U-motor-aw-TL	3 (2-3)	2 (2-3)	0.264
L-U-motor-aw-Amp	5 (4-5.5)	5 (4-7)	0.796
L-U-sensory-TL	2 (2-2)	2 (2-2)	0.317
L-U-sensory-Amp	13.5 (9-19)	14 (7.75-17.25)	0.679
HbA1c	7.1 (6.2-7.8)	6.6 (6.25-7.25)	0.654

Values are stated as median (interquartile range). Counts are provided for gender distribution. *P* values in the last column are from intergroup comparison by Fisher's exact test (for gender), Mann-Whitney *U* test (for other variables). L-M-motor-ew-TL: Terminal latency in the motor component of left median nerve between the elbow and the wrist; L-M-motor-ew-Amp: Amplitude in the motor component of left median nerve between the elbow and the wrist; L-M-sensory-TL: Terminal latency in the sensory component of left median nerve between the elbow and the wrist; L-M-sensory-Amp: Amplitude in the sensory component of left median nerve between the elbow and the wrist; L-U-motor-aw-TL: Terminal latency in the motor component of left ulnar nerve across the wrist; L-U-motor-aw-Amp: Amplitude in the motor component of left ulnar nerve across the wrist; L-U-sensory-TL: Terminal latency and amplitude in the motor component of left ulnar nerve across the wrist; L-U-sensory-Amp: Amplitude in the motor component of left ulnar nerve across the wrist; HbA1c: Glycated hemoglobin.

a greater propensity to proliferate than subcutaneous adipocytes, it is the latter that proliferates following pioglitazone treatment<sup>[12,13]</sup>.

Preadipocyte cell lines like 3T3-L1 and 3T3 F442A manifest miniscule quantum of PPAR- $\gamma$ , but markers of late differentiation, such as aP2, PEPCK, and CAAT/



**Figure 4** Terminal Latency in sensory component of Left Ulnar nerve at baseline and 3 mo (Shaded bar: Pioglitazone; White bar: Non Pioglitazone arm). <sup>a</sup> $P = 0.161$ , <sup>b</sup> $P = 0.038$  (Wilcoxon matched pairs signed rank sum test). L-U-sensory-TL: Terminal Latency in sensory component of Left Ulnar nerve.

enhancer binding protein (C/EBP  $\alpha$ ) is preceded by PPAR- $\gamma$ <sup>[14,15]</sup>. Thiozolidinediones (TZD), Wy-14643 and ETYA assist the transformation of preadipocytes into adipocytes<sup>[16-19]</sup>. Lipid-laden fibroblasts show high PPAR- $\gamma$  expression in diverse fibroblastic lineage (e.g., NIH-3T3, BALB/c-3T3, Swiss-3T3)<sup>[20]</sup>. Adipocyte deposition is a well established pathology in certain metabolic disorders like obesity and it has been shown in pre-clinical studies that PPAR activators promote differentiation of G8 myoblastic cells or transfected C2C12 myoblasts into adipocytes<sup>[21-23]</sup>. Similarly, TZD can differentiate bone marrow stromal cells into adipocytes, analogous to inappropriate adipogenesis that can occur in canine bone marrow<sup>[24,25]</sup>. The c-Cbl-associated protein (CAP) potentiates the phosphorylation of cCbl protooncogene in mature adipocytes, and its expression is accentuated by TZD<sup>[26,27]</sup>.

A multitude of mechanisms have been put forward as the foundation of diabetic polyneuropathy, and therapeutic trials have evaluated the polyol pathway, the advanced glycation end product, protein kinase C, poly ADP-ribose polymerase, and aldose reductase<sup>[28,29]</sup>. The main pathophysiology is an escalation in hyperglycemia induced oxidative stresses and the impairment of anti-oxidative mechanisms in diabetic polyneuropathy<sup>[30]</sup>. TZDs can attenuate oxidative stresses and inflammatory responses<sup>[31]</sup>. Based on these effects, the neuroprotective potential of TZD treatment was investigated in an animal model. These reports explain the neuroprotective effect of TZD by diverse effects of PPAR  $\alpha$  agonist like TNF- $\alpha$  inhibition and IL-6, suppressed protein kinase C (PKC) activity with diminished PKC- $\alpha$  in addition to insulin sensitization<sup>[32-35]</sup>.

In spite of these available data, the clinical impact of this effect of pioglitazone in human subjects has not been studied in detail. Anecdotal data exist about compressive symptoms produced by pioglitazone in the orbit<sup>[4]</sup>. A search undertaken by us in "PubMed" using

keywords viz. "pioglitazone", "carpal tunnel", "compressive neuropathy" yielded no published studies on the effect of pioglitazone therapy on the carpal tunnel. Our study was conceived to address this lacuna in medical literature.

In this case series we evaluated the electrophysiological changes in the left median nerve in the carpal tunnel, in two groups, one receiving pioglitazone and the other not. Both groups received other oral antidiabetic agents, had similar baseline characteristics and achieved similar glycemic control. The ulnar nerve passes superficial to the tunnel in the Guyon's canal, so the left ulnar nerve was also evaluated to assess, the effect of metabolic changes on neural electrophysiology outside the carpal tunnel. It is well known that there is significant association between electrophysiological parameters and metabolic control in diabetes<sup>[36]</sup>. The FBS, PPBS and HbA<sub>1c</sub> were also studied in both the groups to assess whether the changes in diabetes control had an impact on the electrophysiological results in the groups.

We found that a majority, 34 out of 62 (54.84%) of the patients with type 2 diabetes, who underwent NCV testing, although asymptomatic, had electrophysiologically proven CTS. This was in conformity to earlier studies, that demonstrated similarly high prevalence of asymptomatic CTS among patients with diabetes<sup>[12]</sup>.

There was improvement in the amplitude in both motor and sensory components of the median nerve in the non-pioglitazone group at 3 mo. The latter also had electrophysiologically better amplitude in the sensory component of the median nerve compared to pioglitazone group. In the non-pioglitazone group, there was worsening of terminal latency in the motor component of the ulnar nerve, and improvement in the terminal latency of the sensory component in the pioglitazone group. Pioglitazone has favourable effect on nerve electrophysiology which was revealed when the nerve was exposed to compressive neuropathy.

This study had its share of limitations. The sample size is 28 and the observation period was limited to 3 mo in this open label study. However it does generate the hypothesis that patients on pioglitazone are at risk of compressive neuropathy, the pathogenesis of which is established. We were intrigued by the finding that in spite of comparable glycemic control over 3 mo, patients treated with pioglitazone showed superior electrophysiological parameters for the ulnar nerve. The high prevalence of asymptomatic CTS in Indian patients, as found by us, is a novel finding. We are yet to encounter a similar result in published literature. Further studies, ideally randomized controlled trials, are needed to establish the role of pioglitazone in diabetic neuropathy and test our hypothesis.

## COMMENTS

### Background

The carpal tunnel is a fibro-osseous space in the wrist, which also contains small

amounts of adipose tissue. In preclinical studies, pioglitazone, a peroxisome proliferator activator receptor gamma agonist, has been shown to convert pre-adipocytes to adipocytes, although the mechanisms continue to remain elusive. This action has been known to cause compressive symptoms in confined spaces like the orbit in a subgroup of patients. As the carpal tunnel was a closed space with the presence of fatty tissue, it is possible that treatment with pioglitazone could cause delay in the nerve conduction of the median nerve. In order to generate this hypothesis the authors measured terminal latency and amplitude of the motor and sensory components of the median nerve over a fixed distance spanning the wrist and covering the carpal tunnel in patients of type 2 diabetes, at baseline and after 3 mo, in two matched groups of type 2 diabetes patients, one on treatment with pioglitazone and the other without.

## Research frontiers

Pioglitazone has been shown to augment pre-adipocyte proliferation, possibly as a result of cell cycle promoting effect through downregulation of p16(Ink4a) via PPAR. Pioglitazone has also been shown to produce an increase in subcutaneous adipocyte surface. Preclinical studies in rodents have demonstrated that pioglitazone increases whole body adiposity. Although mature visceral adipocytes have a greater propensity to proliferate than subcutaneous adipocytes, it is the latter that proliferates following pioglitazone treatment. In spite of these available data, the clinical impact of this effect of pioglitazone in human subjects has not been studied in detail. Anecdotal data exist about compressive symptoms produced by pioglitazone in the orbit. A search undertaken by us in "PubMed" using keywords viz. "pioglitazone", "carpal tunnel", "compressive neuropathy" yielded no published studies on the effect of pioglitazone therapy on the carpal tunnel. The study was conceived to address this lacuna in medical literature.

## Innovations and breakthroughs

A majority, 34 out of 62 (54.84%) of the patients with type 2 diabetes, who underwent NCV testing, although asymptomatic, had electrophysiologically proven carpal tunnel syndrome. This was in conformity to earlier studies, that demonstrated similarly high prevalence of asymptomatic CTS among patients with diabetes. There was improvement in the amplitude in both motor and sensory components of the median nerve in the non-pioglitazone group at 3 mo. The latter also had electrophysiologically better amplitude in the sensory component of the median nerve compared to the pioglitazone group. In the non-pioglitazone group, there was worsening of terminal latency in the motor component of the ulnar nerve, and improvement in the terminal latency of the sensory component in the pioglitazone group. Pioglitazone thus appeared to have a beneficial effect on nerve electrophysiology which was nullified when the nerve was exposed to entrapment neuropathy. However it does generate the hypothesis that patients on pioglitazone are at risk of compressive neuropathy, the pathogenesis of which is established. The authors were intrigued by the finding that the ulnar nerve showed better electrophysiological parameters in patients who received pioglitazone, although the glycemic control of these patients was similar to those not on pioglitazone. The high prevalence of asymptomatic CTS in Indian patients, as found by the authors, is a novel finding. The authors are yet to encounter a similar result in published literature. Further studies, ideally randomized controlled trials, are needed to establish the role of pioglitazone in diabetic neuropathy and test the authors' hypothesis.

## Applications

The study generates the hypothesis that patients on pioglitazone are at risk of compressive neuropathy, the pathogenesis of which is established. The high prevalence of asymptomatic CTS in Indian patients, as found by the authors, is a novel finding. Further studies, ideally randomized controlled trials, are needed to establish the role of pioglitazone in diabetic neuropathy and test the authors' hypothesis.

## Terminology

L-M-motor-ew-TL: Terminal latency in the motor component of left median nerve between the elbow; L-M-motor-ew-Amp: Amplitude in the motor component of left median nerve between the elbow and the wrist; L-M-sensory-TL: Terminal latency in the sensory component of left median nerve between the elbow and the wrist; L-M-sensory-Amp: Amplitude in the sensory component of left median nerve between the elbow and the wrist; L-U-motor-aw-TL: Terminal

latency in the motor component of left ulnar nerve across the wrist; L-U-motor-aw-Amp: Amplitude in the motor component of left ulnar nerve across the wrist; L-U-sensory-TL: Terminal latency in the sensory component of left ulnar nerve across the wrist; L-U-sensory-Amp: Amplitude in the sensory component of left ulnar nerve across the wrist.

## Peer-review

This is an interesting and well-performed study that reports novel findings regarding the effects of pioglitazone on peripheral nerves and on carpal tunnel syndrome pathogenesis in patients with type 2 diabetes mellitus. The methods are appropriate and the results are clearly presented.

## REFERENCES

- 1 **Tunnel C.** Anatomy of Carpal Tunnel. [Internet]. [accessed 2016 Apr 12]. Available from: URL: [http://www.wheelsonline.com/ortho/anatomy\\_of\\_carpal\\_tunnel](http://www.wheelsonline.com/ortho/anatomy_of_carpal_tunnel)
- 2 **Hasan AU, Ohmori K, Hashimoto T, Kamitori K, Hirata Y, Ishihara Y, Okamoto N, Noma T, Kosaka H, Tokuda M, Kohno M.** Pioglitazone promotes preadipocyte proliferation by downregulating p16(Ink4a). *Biochem Biophys Res Commun* 2011; **411**: 375-380 [PMID: 21741366 DOI: 10.1016/j.bbrc.2011.06.152]
- 3 **Jin HY, Lee KA, Wu JZ, Baek HS, Park TS.** The neuroprotective benefit from pioglitazone (PIO) addition on the alpha lipoic acid (ALA)-based treatment in experimental diabetic rats. *Endocrine* 2014; **47**: 772-782 [PMID: 24532138 DOI: 10.1007/s12020-014-0198-x]
- 4 **Dorkhan M, Lantz M, Frid A, Groop L, Hallengren B.** Treatment with a thiazolidinedione increases eye protrusion in a subgroup of patients with type 2 diabetes. *Clin Endocrinol (Oxf)* 2006; **65**: 35-39 [PMID: 16817816 DOI: 10.1111/j.1365-2265.2006.02542.x]
- 5 **Cherian A, Kuruvilla A.** Electrodiagnostic approach to carpal tunnel syndrome. *Ann Indian Acad Neurol* 2006; **9**: 177-182 [DOI: 10.4103/0972-2327.27665]
- 6 **American Association of Electrodiagnostic Medicine, American Academy of Neurology, American Academy of Physical Medicine and Rehabilitation.** Practice parameter for electrodiagnostic studies in carpal tunnel syndrome: summary statement. *Muscle Nerve* 2002; **25**: 918-922 [PMID: 12115985 DOI: 10.1002/mus.10185]
- 7 **Padua L, Lo Monaco M, Gregori B, Valente EM, Padua R, Tonali P.** Neurophysiological classification and sensitivity in 500 carpal tunnel syndrome hands. *Acta Neurol Scand* 1997; **96**: 211-217 [PMID: 9325471 DOI: 10.1111/j.1600-0404.1997.tb00271.x]
- 8 **Padua L, Lo Monaco M, Valente EM, Tonali PA.** A useful electrophysiologic parameter for diagnosis of carpal tunnel syndrome. *Muscle Nerve* 1996; **19**: 48-53 [PMID: 8538669 DOI: 10.1002/(SICI)1097-4598(199601)19:1<48::AID-MUS6>3.0.CO;2-8]
- 9 **Das Choudhury S, Chatterjee S, Sanyal D, Chakraborti S, Mukherjee A.** The Effect of Pioglitazone on Nerve Conduction Velocity of the Median Nerve in the Carpal Tunnel in Type-2 Diabetes Patients. Oral Papers. *Indian J Pharmacol* [serial online] 2014; **46** Suppl S1: 10-62
- 10 **Koenen TB, Tack CJ, Kroese JM, Hermus AR, Sweep FC, van der Laak J, Stalenhoef AF, de Graaf J, van Tits LJ, Stienstra R.** Pioglitazone treatment enlarges subcutaneous adipocytes in insulin-resistant patients. *J Clin Endocrinol Metab* 2009; **94**: 4453-4457 [PMID: 19820024 DOI: 10.1210/jc.2009-0517]
- 11 **Koh YJ, Park BH, Park JH, Han J, Lee IK, Park JW, Koh GY.** Activation of PPAR gamma induces profound multilocularization of adipocytes in adult mouse white adipose tissues. *Exp Mol Med* 2009; **41**: 880-895 [PMID: 19745605 DOI: 10.3858/emmm.2009.41.12.094]
- 12 **Kajita K, Mori I, Hanamoto T, Ikeda T, Fujioka K, Yamauchi M, Okada H, Usui T, Takahashi N, Kitada Y, Taguchi K, Kajita T, Uno Y, Morita H, Ishizuka T.** Pioglitazone enhances small-sized adipocyte proliferation in subcutaneous adipose tissue. *Endocr J* 2012; **59**: 1107-1114 [PMID: 22972172 DOI: 10.1507/endocrj.EJ12-0259]

- 13 **Dreyer C**, Krey G, Keller H, Givel F, Helftenbein G, Wahli W. Control of the peroxisomal beta-oxidation pathway by a novel family of nuclear hormone receptors. *Cell* 1992; **68**: 879-887 [PMID: 1312391 DOI: 10.1016/0092-8674(92)90031-7]
- 14 **Tontonoz P**, Graves RA, Budavari AI, Erdjument-Bromage H, Lui M, Hu E, Tempst P, Spiegelman BM. Adipocyte-specific transcription factor ARF6 is a heterodimeric complex of two nuclear hormone receptors, PPAR gamma and RXR alpha. *Nucleic Acids Res* 1994; **22**: 5628-5634 [PMID: 7838715 DOI: 10.1093/nar/22.25.5628]
- 15 **Tontonoz P**, Hu E, Spiegelman BM. Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipid-activated transcription factor. *Cell* 1994; **79**: 1147-1156 [PMID: 8001151 DOI: 10.1016/0092-8674(94)90006-X]
- 16 **Lehmann JM**, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM, Kliewer SA. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). *J Biol Chem* 1995; **270**: 12953-12956 [PMID: 7768881 DOI: 10.1074/jbc.270.22.12953]
- 17 **Kletzien RF**, Foellmi LA, Harris PK, Wyse BM, Clarke SD. Adipocyte fatty acid-binding protein: regulation of gene expression in vivo and in vitro by an insulin-sensitizing agent. *Mol Pharmacol* 1992; **42**: 558-562 [PMID: 1435736]
- 18 **Sandouk T**, Reda D, Hofmann C. Antidiabetic agent pioglitazone enhances adipocyte differentiation of 3T3-F442A cells. *Am J Physiol* 1993; **264**: C1600-C1608 [PMID: 8333508]
- 19 **Chawla A**, Schwarz EJ, Dimaculangan DD, Lazar MA. Peroxisome proliferator-activated receptor (PPAR) gamma: adipose-predominant expression and induction early in adipocyte differentiation. *Endocrinology* 1994; **135**: 798-800 [PMID: 8033830 DOI: 10.1210/endo.135.2.8033830]
- 20 **Chen H**, Jackson S, Doro M, McGowan S. Perinatal expression of genes that may participate in lipid metabolism by lipid-laden lung fibroblasts. *J Lipid Res* 1998; **39**: 2483-2492 [PMID: 9831638]
- 21 **Hu E**, Tontonoz P, Spiegelman BM. Transdifferentiation of myoblasts by the adipogenic transcription factors PPAR gamma and C/EBP alpha. *Proc Natl Acad Sci USA* 1995; **92**: 9856-9860 [PMID: 7568232 DOI: 10.1073/pnas.92.21.9856]
- 22 **Teboul L**, Gaillard D, Staccini L, Inadera H, Amri EZ, Grimaldi PA. Thiazolidinediones and fatty acids convert myogenic cells into adipose-like cells. *J Biol Chem* 1995; **270**: 28183-28187 [PMID: 7499310 DOI: 10.1074/jbc.270.47.28183]
- 23 **Grimaldi PA**, Teboul L, Inadera H, Gaillard D, Amri EZ. Transdifferentiation of myoblasts to adipoblasts: triggering effects of fatty acids and thiazolidinediones. *Prostaglandins Leukot Essent Fatty Acids* 1997; **57**: 71-75 [PMID: 9250611 DOI: 10.1016/S0952-3278(97)90495-6]
- 24 **Gimble JM**, Robinson CE, Wu X, Kelly KA, Rodriguez BR, Kliewer SA, Lehmann JM, Morris DC. Peroxisome proliferator-activated receptor-gamma activation by thiazolidinediones induces adipogenesis in bone marrow stromal cells. *Mol Pharmacol* 1996; **50**: 1087-1094 [PMID: 8913339]
- 25 **Deldar A**, Williams G, Stevens C. Pathogenesis of thiazolidinedione induced hematoxicity in the dog. *Diabetes* 1993; **42** Suppl: 179
- 26 **Ribon V**, Johnson JH, Camp HS, Saltiel AR. Thiazolidinediones and insulin resistance: peroxisome proliferator-activated receptor gamma activation stimulates expression of the CAP gene. *Proc Natl Acad Sci USA* 1998; **95**: 14751-14756 [PMID: 9843961 DOI: 10.1073/pnas.95.25.14751]
- 27 **Ribon V**, Printen JA, Hoffman NG, Kay BK, Saltiel AR. A novel, multifunctional c-Cbl binding protein in insulin receptor signaling in 3T3-L1 adipocytes. *Mol Cell Biol* 1998; **18**: 872-879 [PMID: 9447983 DOI: 10.1128/MCB.18.2.872]
- 28 **Evans JL**, Goldfine ID, Maddux BA, Grodsky GM. Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. *Endocr Rev* 2002; **23**: 599-622 [PMID: 12372842 DOI: 10.1210/er.2001-0039]
- 29 **Obrosova IG**, Drel VR, Pacher P, Ilnytska O, Wang ZQ, Stevens MJ, Yorek MA. Oxidative-nitrosative stress and poly(ADP-ribose) polymerase (PARP) activation in experimental diabetic neuropathy: the relation is revisited. *Diabetes* 2005; **54**: 3435-3441 [PMID: 16306359 DOI: 10.2337/diabetes.54.12.3435]
- 30 **Vincent AM**, Russell JW, Low P, Feldman EL. Oxidative stress in the pathogenesis of diabetic neuropathy. *Endocr Rev* 2004; **25**: 612-628 [PMID: 15294884 DOI: 10.1210/er.2003-0019]
- 31 **Giannini S**, Serio M, Galli A. Pleiotropic effects of thiazolidinediones: taking a look beyond antidiabetic activity. *J Endocrinol Invest* 2004; **27**: 982-991 [PMID: 15762051 DOI: 10.1007/BF03347546]
- 32 **Qiang X**, Satoh J, Sagara M, Fukuzawa M, Masuda T, Sakata Y, Muto G, Muto Y, Takahashi K, Toyota T. Inhibitory effect of troglitazone on diabetic neuropathy in streptozotocin-induced diabetic rats. *Diabetologia* 1998; **41**: 1321-1326 [PMID: 9833940 DOI: 10.1007/s001250051072]
- 33 **Yamagishi S**, Ogasawara S, Mizukami H, Yajima N, Wada R, Sugawara A, Yagihashi S. Correction of protein kinase C activity and macrophage migration in peripheral nerve by pioglitazone, peroxisome proliferator-activated-gamma-ligand, in insulin-deficient diabetic rats. *J Neurochem* 2008; **104**: 491-499 [PMID: 17995925 DOI: 10.1111/j.1471-4159.2007.05050.x]
- 34 **Wiggin TD**, Kretzler M, Pennathur S, Sullivan KA, Brosius FC, Feldman EL. Rosiglitazone treatment reduces diabetic neuropathy in streptozotocin-treated DBA/2J mice. *Endocrinology* 2008; **149**: 4928-4937 [PMID: 18583417 DOI: 10.1210/en.2008-0869]
- 35 **Maeda T**, Kiguchi N, Kobayashi Y, Ozaki M, Kishioka S. Pioglitazone attenuates tactile allodynia and thermal hyperalgesia in mice subjected to peripheral nerve injury. *J Pharmacol Sci* 2008; **108**: 341-347 [PMID: 19008646 DOI: 10.1254/jphs.08207FP]
- 36 **Celiker R**, Basgöze O, Bayraktar M. Early detection of neurological involvement in diabetes mellitus. *Electromyogr Clin Neurophysiol* 1996; **36**: 29-35 [PMID: 8654318]

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## Relationship between depression and diabetes in pregnancy: A systematic review

Glynis P Ross, Henrik Falhammar, Roger Chen, Helen Barraclough, Ole Kleivenes, Ian Gallen

Glynis P Ross, Department of Endocrinology, Royal Prince Alfred Hospital, Sydney, New South Wales 2050, Australia

Glynis P Ross, Department of Endocrinology, Bankstown-Lidcombe Hospital, Sydney, New South Wales 2200, Australia

Glynis P Ross, Roger Chen, Sydney Medical School, University of Sydney, Sydney, New South Wales 2006, Australia

Henrik Falhammar, Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, SE 171 76, Sweden

Henrik Falhammar, Department of Endocrinology, Metabolism and Diabetes, Karolinska University Hospital, Stockholm, SE 171 76, Sweden

Henrik Falhammar, Menzies School of Health Research, Royal Darwin Hospital, Darwin, Northern Territory 0811, Australia

Roger Chen, Department of Endocrinology and Metabolism, Concord Repatriation General Hospital, Sydney, New South Wales 2139, Australia

Helen Barraclough, Ole Kleivenes, Lilly Diabetes, Eli Lilly Australia and New Zealand, West Ryde, New South Wales 2114, Australia

Ian Gallen, Diabetes and Endocrinology, Royal Berkshire Foundation Trust, Reading, Berkshire RG1 5BS, United Kingdom

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**Correspondence to:** Dr. Ian Gallen, Diabetes and Endocrinology, Royal Berkshire Foundation Trust, Melrose House, 71 London Road, Reading, Berkshire RG1 5BS, United Kingdom. [ian.gallen@royalberkshire.nhs.uk](mailto:ian.gallen@royalberkshire.nhs.uk)  
**Telephone:** +44-1183-227965  
**Fax:** +44-1183-228810

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## Abstract

### AIM

To systematically review the literature on women with both diabetes in pregnancy (DIP) and depression during or after pregnancy.

### METHODS

In this systematic literature review, PubMed/MEDLINE and EMBASE were searched (13 November 2015) using terms for diabetes (type 1, type 2, or gestational), depression, and pregnancy (no language or date restrictions). Publications that reported on women who had both DIP (any type) and depression or depressive symptoms before, during, or within one year after pregnancy were considered for inclusion. All study types were eligible for inclusion; conference abstracts, narrative reviews, nonclinical letters, editorials, and commentaries were excluded, unless they provided treatment guidance.

### RESULTS

Of 1189 articles identified, 48 articles describing women with both DIP and depression were included (sample sizes 36 to > 32 million). Overall study quality was poor; most studies were observational, and only 12 studies (mostly retrospective database studies) required clinical depression diagnosis. The prevalence of concurrent DIP (any type) and depression in general populations of pregnant women ranged from 0% to 1.6% (median 0.61%; 12 studies). The prevalence of depression among women with gestational diabetes ranged from 4.1% to 80% (median 14.7%; 16 studies). Many studies examined whether DIP was a risk factor for depression or depression was a risk factor for DIP. However, there was no clear consensus for either relationship. Importantly, we found limited guidance on the management of women with both DIP and depression.

### CONCLUSION

Given the increasing prevalence of diabetes and depression, high-quality research and specific guidance for management of pregnant women with both conditions are warranted.

**Key words:** Depression; Diabetes; Postpartum depression; Depressive disorder; Gestational; Diabetes mellitus; Perinatal care; Postnatal care; Pregnancy

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**Core tip:** Depression in women with diabetes in pregnancy (DIP) may be increasingly common. We identified 48 studies of depression and DIP, of variable and often poor quality. The prevalence of concurrent DIP and depression ranged from 0% to 1.6% (median 0.61%; 12 studies). Among women with gestational diabetes, the prevalence of depression ranged from 4.1% to 80% (median 14.7%; 16 studies). There was no clear consensus on whether DIP was a risk factor for depression. Given the increasing prevalence of diabetes and depression, high-

quality research and specific guidance for management of pregnant women with both conditions are warranted.

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## INTRODUCTION

Diabetes affects an increasing number of pregnancies worldwide. In 2015, almost 21 million births (16.2%) were affected by hyperglycemia during pregnancy<sup>[1,2]</sup>. Approximately 10% to 15% of these births involved mothers with pre-existing or newly detected type 1 or type 2 diabetes, with the remaining 85% to 90% being women with gestational diabetes mellitus (GDM)<sup>[1,2]</sup>. As the prevalence of both type 1 and type 2 diabetes in the general population is increasing<sup>[1]</sup>, the number of women affected by diabetes in pregnancy (DIP) is also rising. Indeed, between 2000 and 2010, the age-standardized prevalence of pregnancies in the United States affected by type 1 or type 2 diabetes increased by 37%<sup>[3]</sup> and the prevalence of GDM increased by 56%<sup>[4]</sup>. Diabetes in pregnancy can have adverse effects on both the mother and child, including increased risk of miscarriage, stillbirth, preterm delivery, pre-eclampsia, cesarean section delivery, postpartum development of type 2 diabetes in women with GDM, congenital malformations, fetal macrosomia, neonatal hypoglycemia, neonatal respiratory distress, and obesity and insulin resistance in childhood, followed by impaired glucose tolerance and type 2 diabetes later in life<sup>[1,5,6]</sup>.

Depression during pregnancy or postpartum also adversely affects women and their children. Depression during pregnancy is associated with poorer maternal health, increased likelihood of obstetric complications, preterm birth, and neonatal complications<sup>[5,6]</sup>. Postpartum depression is associated with difficulties with maternal-child bonding, inadequate care of the child, and lower rates of breastfeeding<sup>[7]</sup>.

Recent evidence suggests a bidirectional relationship between diabetes and depression among non-pregnant patients. Several meta-analyses of longitudinal studies suggest that diabetes is a risk factor for the development of depression<sup>[8-10]</sup>. Conversely, depression has been suggested as a risk factor for the development of type 2 diabetes<sup>[11,12]</sup>. In addition, the prevalence of comorbid diabetes and depression is higher than expected, leading to speculation that diabetes and depression may share underlying biological mechanisms<sup>[10,13]</sup>. However, evidence for a link between DIP and depression during pregnancy or postpartum is limited<sup>[5]</sup>. Pregnancy represents a potentially stressful event, which could make women with pre-existing diabetes more vulnerable to

depression. Similarly, a diagnosis of GDM could contribute to depressive symptoms, particularly during pregnancy. Importantly, depression is associated with poor diabetes self-care<sup>[14]</sup>, which may be more challenging during pregnancy and postpartum when diabetes management and glycemic control are especially complex<sup>[15]</sup>. Indeed, women with DIP and depression may struggle to cope with the physical and psychological demands of pregnancy and early motherhood. Given the increasing prevalence of both diabetes and depression among women of childbearing years, the co-occurrence of both conditions during pregnancy or postpartum is likely to become more common. Despite this increase, and the impression among many clinicians that depression in pregnant or postpartum women with diabetes is common, current major guidelines for the treatment and management of DIP<sup>[15-17]</sup> or depression<sup>[18,19]</sup> do not provide adequate advice regarding care of these patients.

The aim of this systematic literature review was to assess the current knowledge regarding the prevalence, treatment, and management of women who have both DIP and depression before, during, or after pregnancy.

## MATERIALS AND METHODS

### Literature search strategy

We searched MEDLINE (PubMed) and EMBASE on 13 November 2015, using Medical Subject Heading (MeSH), Emtree, or free-text terms: (pregnancy OR postpartum period OR pregnant OR postnatal OR post-natal OR antenatal) AND (depression OR depressive disorder, major OR major depression OR depression, postpartum OR puerperal depression OR major depressive disorder OR MDD OR postnatal depression) AND (diabetes mellitus OR diabetes mellitus, type 1 OR diabetes mellitus, type 2 OR diabetes, gestational OR insulin dependent diabetes OR non insulin dependent diabetes OR pregnancy diabetes mellitus OR diabetic OR juvenile diabetes OR type 1 diabetes OR type I diabetes OR insulin-dependent diabetes OR type 2 diabetes OR type II diabetes OR non-insulin dependent diabetes OR NIDDM OR gestational diabetes). Searches were tailored to each database and restricted to human studies. There were no restrictions on publication date, publication type, or language.

### Eligibility criteria

Publications that reported on women who had both DIP (type 1, type 2, or GDM) and depression or depressive symptoms before, during, or within one year after pregnancy were considered for inclusion. All study types were eligible for inclusion, including meta-analyses, systematic reviews, randomized and nonrandomized clinical trials, observational studies (prospective and retrospective), case reports, clinical practice guidelines, and other publications providing guidance on diagnosis, treatment, or management.

Publications were excluded if they described studies

not conducted in humans, studies in which data for women with DIP and depression were pooled with data for women with other conditions, studies that reported depressive symptoms based on measures of anxiety or bipolar disorder, or studies that only reported fetal or newborn outcomes (*i.e.*, no maternal outcomes or prevalence data). Conference abstracts, narrative reviews, systematic reviews that did not report original data, nonclinical letters, editorials, and commentaries were excluded, unless they provided treatment guidance.

### Study selection and data extraction

One person (medical writer contracted by Eli Lilly and Company) conducted the literature search and screened the titles and abstracts of retrieved publications using the predefined eligibility criteria. The full text of publications identified for potential inclusion were rescreened using the same criteria. Reference lists of reviews and other relevant publications were screened to identify additional publications. All authors reviewed and approved the publications identified for inclusion.

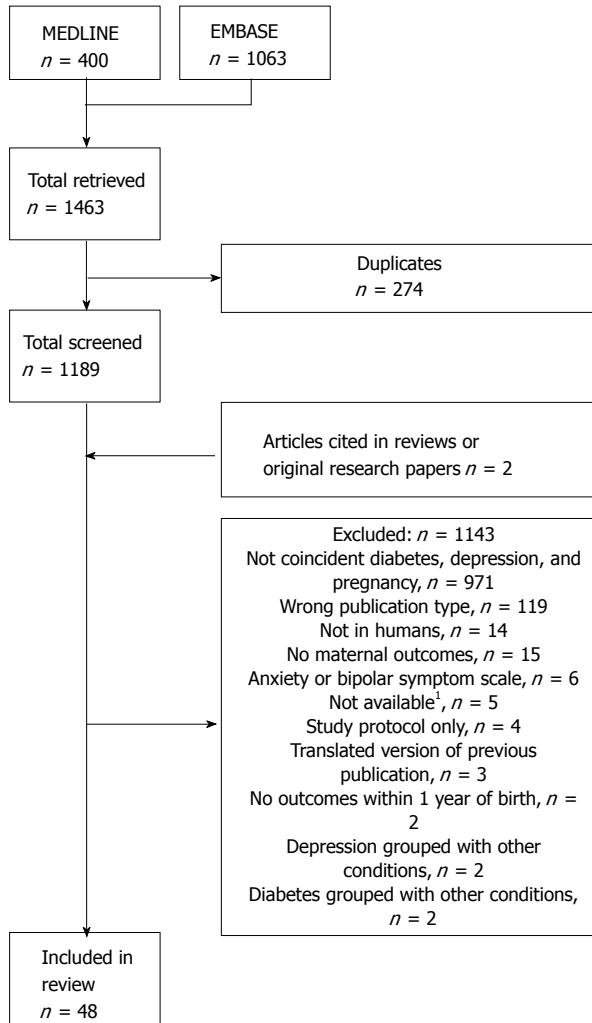
The medical writer extracted all relevant data, including publication type and year, study design, study objectives, country of origin, sample size, patient characteristics, diabetes type(s), definition or measures of depression, and main outcomes, from the included publications. The risk of bias was assessed by study quality components (study design, sample size, outcomes) and by the depression and diabetes definitions used in each study. Because information on this topic is lacking, all levels of evidence were included in the review.

Outcome measures included: Incidence/prevalence of DIP and depression among pregnant or postpartum women; relationship between DIP and depression; relative risk of developing depression during or after pregnancy among women with DIP vs pregnant women without diabetes; relative risk of developing GDM among women with depression vs women without depression; clinical or demographic factors related to increased risk of having both DIP and depression during or after pregnancy; methods of diagnosis or measurement of depression; and treatment/management strategies.

## RESULTS

### Literature search results

A total of 1463 publications were retrieved from MEDLINE and EMBASE; after removal of duplicates, 1189 publications were screened (Figure 1). Of these, 46 publications were selected for inclusion<sup>[20-65]</sup>. Manual screening of bibliographies identified two additional relevant studies<sup>[66,67]</sup>. Overall, 48 publications were included in this review (Figure 1, Tables 1-3, Supplementary Table 1). Of these, 30 described prospective observational studies<sup>[20,21,23,24,26,27,29-31,34,35,37,38,41,43-45,47-49,51,54,57,58,60-62,65-67]</sup>, 15 described retrospective observational studies<sup>[22,25,28,32,39,40,42,46,50,53,55,56,59,63,64]</sup>, and three described



**Figure 1 Publication flow diagram.** <sup>1</sup>Unavailable articles were unlikely to be relevant based on the title and/or abstract.

randomized controlled trials (RCTs)<sup>[33,36,52]</sup>, two of which reported only baseline data<sup>[36,52]</sup>. Two publications described the same study, but reported different subgroup analyses<sup>[23,29]</sup>.

### Overview of study characteristics

A total of 28 studies included only women with GDM<sup>[20,23,26-29,31,33,35-38,40,43-45,47-49,51-53,57,58,60,61,63,64]</sup>, 14 included women with either GDM or pre-existing diabetes (although the type was not always reported)<sup>[22,25,39,41,42,46,50,54-56,59,62,65,66]</sup>, one included women with either GDM or type 1 diabetes<sup>[34]</sup>, one included only women with type 1 diabetes<sup>[67]</sup>, one included only women with pre-existing diabetes (type not reported)<sup>[30]</sup> and three did not report the type of diabetes<sup>[21,24,32]</sup> (Tables 1-3, Supplementary Table 1). Sample sizes ranged from 36<sup>[65]</sup> to more than 32 million in a retrospective analysis of a nationwide hospital database<sup>[22]</sup> (Tables 1-3, Supplementary Table 1).

### Study quality

Overall study quality was poor. Most studies were prospective observational studies (Tables 1-3, Supple-

mentary Table 1), which were subject to limitations such as small sample size and selection bias. Further, most studies defined depression using measures of depressive symptoms rather than more rigorous clinical diagnosis tools. Among those that did use clinical diagnosis tools, most were retrospective, including six national, state/provincial, or veterans' health database studies<sup>[32,40,50,55,56,64]</sup>, two claims registry studies<sup>[39,46]</sup>, and three hospital records review studies<sup>[22,28,63]</sup>. Although these studies were large, their retrospective nature was an inherent limitation. Unlike the health database studies, the claims registry and hospital records review studies were subject to potential selection bias. Importantly, the primary objective of many of the studies was not relevant to this systematic review (Supplementary Table 1), and the results we collected were often secondary or incidental findings.

The small number of RCTs identified may reflect ethical concerns regarding enrolment of pregnant women in interventional studies. The one completed RCT was the highest quality study included in this review<sup>[33]</sup>, having appropriate allocation sequence generation and concealment, as well as attempts to maintain blinding; however, Edinburgh Postnatal Depression Scale (EPDS) data at 3 mo postpartum were available for fewer than 60% of patients, indicating potential attrition bias.

### Definition of depression

The definition of depression varied widely across the studies (Tables 1-3), and only a quarter of the studies (almost all retrospective) classified participants as having depression based on a formal clinical diagnosis. Only one prospective study defined depression using the Structural Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV)<sup>[60]</sup>. This study examined responses to oral glucose challenge tests among women with or without current or past diagnosis of a psychiatric disorder, including major depressive disorder. However, only 3 of 186 women were subsequently diagnosed with GDM, and the publication did not report whether these women had depression, another psychiatric disorder, or no psychiatric disorder. Eleven retrospective studies used International Classification of Diseases (ICD) codes<sup>[68]</sup> in medical records to classify participants as having current or a history of depression<sup>[22,28,32,39,40,46,50,55,56,63,64]</sup>. One of these retrospective studies also included diagnoses based on the DSM-IV<sup>[50]</sup>. One retrospective study<sup>[59]</sup> and one prospective study<sup>[62]</sup> relied on participant self-reporting of depression diagnosis. Aside from these studies, all other studies used measures of depressive symptoms, most commonly the EPDS; however, the cut-off score for clinically significant depression varied from 9 to 15. Other depressive symptom scales included the Beck Depression Inventory, the Centre for Epidemiologic Studies Depression scale, the Montgomery-Åsberg Depression Rating Scale, and the Patient Health Questionnaire.

In general, the large retrospective studies that used ICD codes reported a significant association between



**Table 1 Outcomes of included studies involving women with gestational diabetes**

First author, study design	Definition/ measures of depression	Timing of depression measures	Overall, <i>n</i> subgroups, <i>n</i>	Main outcomes/findings
Abdollahi <sup>[20]</sup> Prospective, cohort	EPDS $\geq 12$	Within 12 wk after delivery	<i>n</i> = 1449	Women with GDM had greater risk of postpartum depression than women without GDM [adjusted OR (95%CI): 2.93 (1.46-5.88), <i>P</i> = 0.002]
<sup>1</sup> Bener <sup>[23]</sup> Prospective, cross-sectional	EPDS $\geq 12$	Within 6 mo after delivery	<i>n</i> = 1379 With depression, <i>n</i> = 243; Without depression, <i>n</i> = 1136	Prevalence of GDM was numerically, but not statistically, higher in women with depression (9.9%) <i>vs</i> women without depression (6.2%) ( <i>P</i> = 0.051)
Berger <sup>[25]</sup> Retrospective	EPDS $\geq 13$ or did not answer "No" to self-harm question	Within 4 d after delivery	Unselected, <i>n</i> = 322 History of mental illness, <i>n</i> = 215	In the unselected group, prevalence of GDM was higher in women with postpartum depression (27.3%) <i>vs</i> women without depression (9.0%) ( <i>P</i> = 0.04); there was no difference in the group with previous mental illness (19.4% <i>vs</i> 10.2%, <i>P</i> = 0.14) In the unselected group, GDM was associated with postpartum depression [OR (95%CI): 12.1 (1.9-77.8)] In the unselected group, overall prevalence of depression and GDM was 0.9% (3 of 322)
Bisson <sup>[26]</sup> Prospective, case-control	EPDS $\geq 10$	Approx. 30 wk gestation	<i>n</i> = 52 GDM, <i>n</i> = 26; No GDM, <i>n</i> = 26	Women with GDM had a greater prevalence of depressive symptoms <i>vs</i> women without GDM (23% <i>vs</i> 0%, <i>P</i> = 0.023) Mean (SD) EPDS score was 6.8 (4.0) for women with GDM and 4.2 (2.6) for women without GDM ( <i>P</i> < 0.05)
Blom <sup>[27]</sup> Prospective	EPDS > 12	2 mo after delivery	<i>n</i> = 4941 With depression, <i>n</i> = 396; Without depression, <i>n</i> = 4545	No significant difference in the proportion of women with GDM between those who did (4/396; 1.0%) and did not (28/4545; 0.6%) have depression ( <i>P</i> $\geq$ 0.05) Calculated prevalence of women with both GDM and depression = 0.08% (4/4941)
Bowers <sup>[28]</sup> Retrospective	ICD9 codes 296.2, 296.3, and 311	Coded on medical history or hospital discharge record	<i>n</i> = 128295 With depression, <i>n</i> = 5815 (medical history, <i>n</i> = 5350); Without depression, <i>n</i> = 122480	Women with history of depression were more likely to have GDM than women without history of depression (5.4% <i>vs</i> 4.3%; <i>P</i> value NR) Depression was associated with significantly increased risk of GDM [OR (95%CI): adjusted for age, race/ethnicity, study site, insurance, and parity: 1.42 (1.26-1.60)]; similar results when restricted to women with history of pre-pregnancy depression [adjusted OR (95%CI): 1.36 (1.20-1.54)] Calculated prevalence of coincident GDM and depression was 313 of 128295 (0.24%)
<sup>1</sup> Burgut <sup>[29]</sup> Prospective, cross-sectional	EPDS $\geq 12$	Within 6 mo of delivery	<i>n</i> = 1379 Qatari women, <i>n</i> = 837 Other Arab women, <i>n</i> = 542 With depression, <i>n</i> = 243 With history of diabetes, <i>n</i> = 310	GDM increased risk of depression in Qatari women [adjusted OR (95%CI): 1.65 (1.02-2.69)], but not in other Arab women [1.09 (0.63-1.91)]
Chazotte <sup>[31]</sup> Prospective	CES-D $\geq 16$	Weeks 34-36 of gestation	<i>n</i> = 90 GDM, <i>n</i> = 30; High risk of preterm birth, <i>n</i> = 30	56.7% of women with GDM had CES-D $\geq 16$ ; this was not significantly different <i>vs</i> women at low (33.3%) or at high (70%) risk of preterm birth ( <i>P</i> $\geq$ 0.05) Mean (SD) CES-D score was 17.0 (9.1) for women with GDM, 20.9 (9.4) for women at high risk of preterm birth, and 13.7 (7.5) for women at low risk of preterm birth ( <i>P</i> $\geq$ 0.05)
Crowther <sup>[33]</sup> RCT	EPDS $\geq 12$	3 mo after delivery	Low risk of preterm birth, <i>n</i> = 30 <i>n</i> = 1000 Intervention <sup>2</sup> , <i>n</i> = 490; Routine care, <i>n</i> = 510	Significantly lower proportion of women in the intervention group (8%; 23/278 respondents) had EPDS indicative of depression <i>vs</i> women in the routine care group (17%; 50/295 respondents) ( <i>P</i> = 0.001)
Dalfra <sup>[34]</sup> Prospective	CES-D $\geq 16$	3rd trimester and 8 wk after delivery	<i>n</i> = 245 GDM, <i>n</i> = 176 (treated with diet, <i>n</i> = 109; treated with insulin, <i>n</i> = 68); No DM, <i>n</i> = 39	Mean (SD) CES-D scores at 3 <sup>rd</sup> trimester were 17.0 (8.6) among women with GDM and 18.0 (8.7) among women without DM ( <i>P</i> = 0.52) Mean (SD) CES-D scores at 3 <sup>rd</sup> trimester were 16.6 (8.1) among women with GDM treated with diet and 17.7 (9.4) among women with GDM treated with insulin ( <i>P</i> = 0.58) The severity of depressive symptoms increased from the 3 <sup>rd</sup> trimester to after delivery in women with GDM [estimated mean difference in CES-D score (95%CI): 5.7 (4.2-7.3)], but decreased in women without DM [-2.7 (-5.9-0.5); <i>P</i> < 0.0001 between groups]
Daniells <sup>[35]</sup> Prospective, longitudinal	MHI-5 $\geq 16$	Weeks 30 and 36 of gestation, and 6 wk after delivery	<i>n</i> = 100 GDM, <i>n</i> = 50; No GDM, <i>n</i> = 50	Significantly higher proportion of women with GDM (30%) were depressed at Week 30 <i>vs</i> women who did not have GDM (12%) [OR (95%CI): 3.14 (1.1-8.94), <i>P</i> = 0.03]; however, there was no difference at Week 36 or after delivery ( <i>P</i> $\geq$ 0.05) Mean (SD) MHI-5 scores: Week 30: GDM, 13.9 (4.8); no GDM, 11.4 (3.8), <i>P</i> = 0.004; Week 36: GDM, 10.9 (3.8); no GDM, 11.7 (4.0), <i>P</i> = 0.31; postpartum: GDM, 11.5 (4.5); no GDM, 11.7 (4.0), <i>P</i> = 0.79 No significant difference in MHI-5 scores in women who were being treated with insulin ( <i>n</i> = 7) compared with those being managed with diet only ( <i>P</i> = 0.06; MHI-5 scores NR)

de Wit <sup>[36]</sup> Analysis of baseline RCT data	WHO-5 < 50	Early pregnancy (< 20 wk)	<i>n</i> = 98 obese women Depressed, <i>n</i> = 26	Prevalence of GDM was 13.5% of total sample of obese women and 19.2% of the subgroup with depression (NS; <i>P</i> value NR)
Ertel <sup>[37]</sup> Prospective, cohort	EPDS ≥ 15	Early pregnancy (< 20 wk)	<i>n</i> = 934	No significant association between depressive symptoms in early pregnancy and GDM measures at mid-pregnancy [adjusted OR (95%CI): for abnormal glucose tolerance associated with depression: 1.34 (0.81-2.23); for impaired glucose tolerance associated with depression: 1.53 (0.73-3.22)]
Huang <sup>[38]</sup> Prospective, cohort	EPDS ≥ 13	Mid- pregnancy (median 27.9 wk) and 6 mo (median 6.5 mo) after delivery	Prenatal, <i>n</i> = 2112 Postpartum, <i>n</i> = 1686	Prevalence of GDM was 8% among women with prenatal depression, 6% among women without prenatal depression, 7% among women with postpartum depression, and 5% among women without postpartum depression Compared with women with normal glucose tolerance, the odds of prenatal depression were significantly higher in women with isolated hyperglycemia [adjusted OR (95%CI): 1.80 (1.08-3.00)], but not in women with impaired glucose tolerance [1.43 (0.59-3.46)] or GDM [1.45 (0.72-2.91)] There was a 25% higher odds of prenatal depression per SD increase (27 mg/dL) in glucose levels [OR (95%CI): 1.25 (1.07-1.48)] Pregnancy hyperglycemia was not associated with significantly higher odds of postpartum depression Prevalence of depression among women with GDM was 5.3% Relative risk (95%CI): of depression in women with GDM <i>vs</i> women with no DM was 1.17 (1.12-1.21) Prevalence of concurrent GDM and depression was 0.4%
Jovanovic <sup>[39]</sup> Retrospective, claims database	ICD-9 codes 311, 296.2, 296.3, 300.4, 301.12, 309.1	Not specified, but data spanned from 21 mo before to 3 mo after delivery	<i>n</i> = 839792 GDM, <i>n</i> = 52848 No DM, <i>n</i> = 773751	Prevalence of depression among women with GDM was 5.3% Relative risk (95%CI): of depression in women with GDM <i>vs</i> women with no DM was 1.17 (1.12-1.21) Prevalence of concurrent GDM and depression was 0.4%
Katon 2011 <sup>[41]</sup> Cross-sectional analysis of prospective cohort	PHQ-9	3 <sup>rd</sup> trimester	<i>n</i> = 2398 GDM, <i>n</i> = 425; No DM, <i>n</i> = 1747	Prevalence (95%CI): of probable major depression among women with GDM was 4.5% (2.5%-6.4%) by PHQ-9 score, 5.7% (3.5%-7.9%) by antidepressant use, and 8.7% (6.0%-11.4%) by either PHQ-9 or antidepressant use, compared with the prevalence among women without DM [PHQ-9: 4.1% (3.2%-5.1%); antidepressants: 6.2% (5.1%-7.3%); PHQ-9 and antidepressants: 9.6% (8.2%-11.0%)] After adjusting for demographic characteristics, chronic medical conditions, and pregnancy variables, GDM was not associated with major [OR (95%CI): 0.90 (0.61-1.32)] or any [OR (95%CI): 0.95 (0.68-1.33)] antenatal depression Prevalence of depression was 9.3% in women with GDM and 8.8% in women without DM (no statistical analysis)
Katon 2014 (VA) <sup>[40]</sup> Retrospective, VA database	ICD-9 codes 296.2-296.39	Up to date of delivery	<i>n</i> = 2288 GDM, <i>n</i> = 118 No GDM or hypertensive disorder, <i>n</i> = 1966	Prevalence of depression was 9.3% in women with GDM and 8.8% in women without DM (no statistical analysis)
Katon 2014 (PPD) <sup>[42]</sup> Retrospective, hospital database	PHQ-9	2nd or 3rd trimester and 6 wk after delivery	<i>n</i> = 1423	Prevalence of GDM did not differ between women with postpartum depression (19.3%) and women without postpartum depression (20.7%) ( <i>P</i> = 0.89) GDM was not a risk factor for postpartum depression [OR (95%CI): 0.68 (0.40-1.13), <i>P</i> = 0.13] Prevalence of concurrent GDM and depression was 1.12% Prevalence of depression did not differ between women with GDM (80%) and women without GDM (83%) ( <i>P</i> = 0.4)
Keskin <sup>[43]</sup> Prospective, cohort	BDI ≥ 17	24-28 wk gestation	<i>n</i> = 89 GDM, <i>n</i> = 44 No GDM, <i>n</i> = 45	No difference in the proportion of women with depressive symptoms in the GDM (14.1%) <i>vs</i> no GDM (13.5%) group ( <i>P</i> > 0.05) After adjustment, GDM was not associated with an increase in depressive symptoms between pregnancy and postpartum [adjusted OR (95%CI): 1.22 (0.54-2.77)] Calculated prevalence of both GDM and depression = 0.62%
Kim <sup>[44]</sup> Prospective, longitudinal	CES-D (cut- off NR)	Week 12-20 of gestation and 8-12 wk after delivery	<i>n</i> = 1445 GDM, <i>n</i> = 64; No GDM, <i>n</i> = 1233	Women with GDM who participated in a 4-wk educational coaching program had a greater decrease in depression scores [mean (SD) change from baseline: -3.77 (6.50)] than women with GDM who did not participate in the program [mean (SD) change from baseline: 1.23 (6.76)] ( <i>P</i> = 0.043)
Ko <sup>[45]</sup> (Korean) Prospective, cohort	Postpartum depression model (dissertation by Ji Bae, Ewha Womans University)	Weeks 24 and 28 of gestation	<i>n</i> = 68 Coaching program group, <i>n</i> = 34 Control group, <i>n</i> = 34	Prevalence of depression in women with GDM taking insulin was 16.0% <i>vs</i> 13.7% among women with GDM not taking insulin ( <i>P</i> value not reported) Relative to women without diabetes, risk of depression was higher in both women with GDM taking insulin [adjusted OR (95%CI): 1.85 (1.19-2.87)] and in women with GDM not taking insulin [adjusted OR (95%CI): 1.69 (1.09-2.62)]
Kozhimannil <sup>[46]</sup> Retrospective, cohort	ICD9 codes 296.2, 296.3, 300.4, 301.12, 309.1, and 311	During the 6 mo before and up to 1 yr after delivery	<i>n</i> = 11024 With GDM, <i>n</i> = 346 (taking insulin, <i>n</i> = 163); No DM, <i>n</i> = 10367	Prevalence of depression in women with GDM taking insulin was 16.0% <i>vs</i> 13.7% among women with GDM not taking insulin ( <i>P</i> value not reported) Relative to women without diabetes, risk of depression was higher in both women with GDM taking insulin [adjusted OR (95%CI): 1.85 (1.19-2.87)] and in women with GDM not taking insulin [adjusted OR (95%CI): 1.69 (1.09-2.62)]

Levy-Shiff <sup>[66]</sup> Prospective	BDI	2 <sup>nd</sup> trimester	<i>n</i> = 153 GDM, <i>n</i> = 51 No DM, <i>n</i> = 49	No significant difference in depression during 2 <sup>nd</sup> trimester between GDM [mean (SD) BDI score 6.70 (4.46)] and controls [6.59 (5.88), <i>P</i> ≥ 0.05] For sample as a whole, higher levels of cognitive assessment of pregnancy as a challenge was associated with lower depression ( <i>P</i> < 0.05) Prevalence of GDM was 7.6% in white ( <i>P</i> < 0.05 <i>vs</i> all other ethnic groups), 14.9% in Asian/Pacific Islander ( <i>P</i> < 0.05 <i>vs</i> other ethnic groups), 10.1% in Hispanic ( <i>P</i> < 0.05 <i>vs</i> white and Asian/Pacific Islander groups), and 10.1% in black ( <i>P</i> < 0.05 <i>vs</i> white and Asian/Pacific Islander groups) populations Prevalence of pre-existing depression was 2.8% in white ( <i>P</i> < 0.05 <i>vs</i> all other ethnic groups), 12.4% in Asian/Pacific Islander ( <i>P</i> < 0.05 <i>vs</i> all other ethnic groups), 7.6% in Hispanic ( <i>P</i> < 0.05 <i>vs</i> all other ethnic groups), and 5.5% in black ( <i>P</i> < 0.05 <i>vs</i> all other ethnic groups) populations No association between GDM and PPD; African Americans with GDM had decreased likelihood of PPD compared with those without GDM [OR (95%CI): 0.1 (0.0-0.5)] Weighted percentage of women with PPD with or without GDM was 10% <i>vs</i> 7.5% in white women ( <i>P</i> < 0.05), 18.6% <i>vs</i> 14.4% in Asian/Pacific Islander ( <i>P</i> ≥ 0.1), 13.8% <i>vs</i> 9.8% in Hispanic ( <i>P</i> ≥ 0.1), and 1.1% <i>vs</i> 10.4% in black women ( <i>P</i> ≥ 0.1) Proportion of patients with major depressive episode who also had GDM was 2.6% (same as overall population, which was 2.7%)
Liu <sup>[47]</sup> Prospective	Survey asking if diagnosed or discussed with HCP	Postpartum (mean 9.7 mo)	<i>n</i> = 3748 White, <i>n</i> = 1043 Asian/Pacific Islander, <i>n</i> = 425 Hispanic, <i>n</i> = 1253 Black, <i>n</i> = 1027	Mean (SD) EPDS scores in late pregnancy [7.55 (5.48)], immediately postpartum [7.00 (3.74)], and 3-4 mo postpartum [6.36 (5.63)] were not different in women with GDM compared with women without pregnancy complications [mean (SD) EPDS scores 6.41 (4.37), 4.69 (4.43), and 5.48 (4.88) in late pregnancy, immediately postpartum, and 3-4 mo postpartum, respectively] ( <i>P</i> ≥ 0.05) Prevalence of GDM during the index pregnancy was 3.4% in women with pre-pregnancy depression and 4.7% in women with no known mental illness (no statistical analysis) Prevalence of GDM and pre-pregnancy depression was 0.029%
Manoudi <sup>[48]</sup> Prospective, cross-sectional	MINI; HAM-D	NR	<i>n</i> = 187 GDM 2.7%	
Mautner <sup>[49]</sup> Prospective	EPDS	24 <sup>th</sup> -37 <sup>th</sup> week of gestation; 2-5 d postpartum; 3-4 mo postpartum	<i>n</i> = 40 GDM, <i>n</i> = 11 No GDM, <i>n</i> = 29	
Mei-Dan <sup>[50]</sup> Retrospective, health administration database	ICD-9, ICD-10CA, and/or DSM-IV (ICD codes NR)	Within 5 yr before pregnancy	<i>n</i> = 437941 With pre-pregnancy depression, <i>n</i> = 3724 No known mental illness, <i>n</i> = 432358	
Natasha <sup>[51]</sup> Prospective, case-control	MADRS ≥ 13	Approx. 25 wk gestation	<i>n</i> = 748 GDM, <i>n</i> = 382 No GDM, <i>n</i> = 366	Prevalence of depression was higher in women with GDM (25.92%) than in women without GDM (10.38%) ( <i>P</i> value NR) There were significant associations between depression and current GDM ( <i>P</i> < 0.001) and between depression and a history of GDM ( <i>P</i> < 0.018) Mean (variance) MADRS scores were significantly higher in women with GDM [8.33 (7.23)] than women without GDM [4.42 (5.89)] ( <i>P</i> value NR) Relative to women without GDM, women with GDM were more likely to have mild (MADRS score 13-19; adjusted OR: 3.07 or 4.06) <sup>3</sup> or moderate (MADRS score 20-34; adjusted OR: 3.94) depression ( <i>P</i> < 0.001) 24 (34%) women with GDM had EPDS > 9 at postpartum visit [mean (SD) score 11.4 (2.2)]; cesarean delivery ( <i>P</i> = 0.005) and greater gestational weight gain ( <i>P</i> = 0.035), but not history of depression ( <i>P</i> = 0.97), were associated with PPD
Nicklas <sup>[52]</sup> Baseline description of RCT cohort	EPDS > 9	Mean (SD) 7.0 (1.7) wk postpartum (range, 4-15 wk)	<i>n</i> = 71	
O'Brien <sup>[53]</sup> Retrospective, records review	EPDS ≥ 10	Mean (SD) 13.6 (8.2) wk gestation	<i>n</i> = 362 With depression, <i>n</i> = 256 Without depression, <i>n</i> = 106	No difference in prevalence of GDM between women with EPDS < 10 (14.6%) and those with EPDS ≥ 10 (15.0%) ( <i>P</i> ≥ 0.05)
Ragland <sup>[54]</sup> Prospective, cross-sectional	BDI > 13	During pregnancy	<i>n</i> = 50 GDM, <i>n</i> = 22	Mean BDI score among women with GDM was 13.7 9 (41%) women with GDM had BDI > 13
<sup>4</sup> Räisänen 2013 <sup>[56]</sup> Retrospective, registry review	ICD10 codes F31.3, F31.5, F32-34	Up to 6 wk postpartum or a history of depression	<i>n</i> = 511422	Prevalence of GDM: 11.2% of women without any depression ( <i>n</i> = 492103), 13.8% of women with history of depression but not PPD ( <i>n</i> = 17881), 17.4% of women with PPD but no history of depression ( <i>n</i> = 431), and 17.6% of women with both history of depression and PPD ( <i>n</i> = 1007) ( <i>P</i> ≤ 0.001) Among women with history of depression, increased prevalence of PPD was associated with GDM [OR (95%CI): 1.62 (1.23-2.14)]
<sup>4</sup> Räisänen 2014 <sup>[55]</sup> Retrospective, registry review	ICD10 codes F31.3, F31.5, F32-34	Up to hospital discharge after delivery	<i>n</i> = 511938	Prevalence of GDM: 11.2% of women without any depression ( <i>n</i> = 493037), 13.4% of women with history of depression but not during pregnancy ( <i>n</i> = 14781), 14.5% of women with depression during pregnancy but no history of depression ( <i>n</i> = 2189), and 17.6% of women with both depression during pregnancy and history of depression ( <i>n</i> = 1931) ( <i>P</i> ≤ 0.001) An increased prevalence of depression during pregnancy was associated with GDM [adjusted OR (95%CI): 1.29 (1.11-1.50)]

Rumbold <sup>[57]</sup> Prospective	EPDS $\geq 12$	Late pregnancy (for GDM)	$n = 212$ GDM (or glucose intolerance of pregnancy), $n = 25$ Negative OGCT, $n = 95$ Positive OGCT/negative OGTT, $n = 29$	No difference in proportion of women with EPDS score $\geq 12$ in the GDM group (19%) compared with other groups ( $P \geq 0.05$ )
Silveira <sup>[58]</sup> Prospective, cohort	EPDS $\geq 13$	Early (mean 12.4 wk gestation) and mid (mean 21.3 wk) pregnancy	$n = 1115$ GDM, $n = 52$ No glucose abnormality, $n = 953$	Prevalence of GDM did not differ between women with at least minor depression (EPDS $\geq 13$ ) and women without depression (4.6% vs 5.6%) ( $P = 0.58$ ) Prevalence of GDM did not differ between women with probably major depression (EPDS $\geq 15$ ) and women without major depression (4.1% vs 5.6%) ( $P = 0.51$ )
Singh <sup>[59]</sup> Retrospective	BDI $\geq 10$ ; self-reported medical history	During pregnancy	$n = 152$ History of depression, $n = 39$ No history of depression, $n = 113$	Of 39 women with history of depression, 15 (38%) had GDM Of 113 women with no history of depression, 67 (59%) had GDM ( $P$ value not reported)
Sit <sup>[60]</sup> Prospective	DSM-IV (SCID)	Past or current diagnosis	$n = 186$ Past MDD, $n = 41$ Current MDD, $n = 39$ Bipolar disorder, $n = 45$ No psychiatric disorder, $n = 61$	Mean (SD) glucose concentration after OGCT was 100 (25.0) mg/dL and did not differ among groups ( $P = 0.564$ ) Rate of abnormal OGCT was 7% (13 of 186) and did not differ among the groups ( $P = 1.000$ ) Only 3 women with abnormal OGCT were confirmed as having GDM (group not specified)
Song <sup>[61]</sup> (Chinese) Prospective	Self-rating Depression Scale $\geq 41$	During pregnancy	$n = 104$ GDM, $n = 50$ No GDM, $n = 54$	Incidence of depression was 22% in women with GDM, significantly higher than in women without GDM (7.4%) ( $P < 0.05$ ) Among women with GDM, mean (SD) insulin concentration 1 h after OGTT was significantly lower in women with depression [58.3 (32.4) mIU/mL, $n = 11$ ] than in those without depression [102.1 (65.2) mIU/mL, $n = 39$ ] ( $P < 0.05$ )
Sundaram <sup>[62]</sup> Prospective, exploratory	Survey of PPD diagnosis; survey of symptoms based on PHQ-2	Postpartum	Up to 61733 pregnancies	In analysis of data from 22 states, GDM was not a significant predictor of PPD symptoms [OR (95%CI): 1.13 (0.93-1.30), $n = 45642$ , $P = 0.14$ ] or diagnosis [OR (95%CI): 0.96 (0.64-1.52), $n = 5919$ , $P = 0.89$ ]
Walmer <sup>[63]</sup> Retrospective, electronic medical records	ICD-9 codes 296.2, 296.3, 309.0, 309.1, 311, 300.4	Postpartum	$n = 18888$ pregnancies (14988 women) GDM, $n = 696$ pregnancies (659 women)	After adjusting for age, pre-eclampsia, and preterm birth, GDM was significantly associated with increased risk of PPD [adjusted OR (95%CI): 1.46 (1.16-1.83), $P = 0.001$ ]; however, the association was not significant after adjusting for other clinical and demographic characteristics [adjusted OR (95%CI): 1.29 (0.98-1.70), $P = 0.064$ ] In subanalyses of ethnic/racial groups, GDM was significantly associated with PPD in black and white women, but not Hispanic women, after adjusting for age, pre-eclampsia, and preterm birth; the associations were not significant after full adjustment GDM was significantly predictive of mental health disorder (including depression, anxiety, and others) within 3 mo postpartum [adjusted OR (95%CI): 1.38 (1.04-1.85), $P = 0.028$ ]
Whiteman <sup>[64]</sup> Retrospective, maternal and infant database	ICD-9-CM codes 293.83, 296.2, 296.3, 300.4, 301.12, 309.0, 309.1, 311	Up to hospital discharge after delivery	$n = 1057647$	GDM was significantly associated with increased risk of depression [adjusted OR (95%CI): 1.44 (1.26-1.65)] ( $P$ value NR) Obesity was also associated with increased risk of depression, but there was no significant, additive interaction between GDM and obesity

<sup>1</sup>The Bener *et al* and Burgut *et al* publications describe the same study, although different subgroups analyses are reported; <sup>2</sup>Intervention comprised dietary advice, blood glucose monitoring, insulin therapy as needed, and usual care; <sup>3</sup>Note that the adjusted OR for mild depression is variously reported as 3.065062 or 4.06 in the publication; <sup>4</sup>The Räisänen *et al* 2013 and 2014 publications use the same database within the same time period (2002-2010) and, therefore, the study populations are almost identical. BDI: Beck Depression Inventory; CES-D: Center for Epidemiologic Studies Depression scale; CI: Confidence interval; DM: Diabetes mellitus; DSM-IV: Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; EPDS: Edinburgh Postnatal Depression Scale; GDM: Gestational diabetes mellitus; HAM-D: Hamilton Depression Scale; HCP: Healthcare professional; ICD: International Classification of Diseases; ICD-9-CM: International Classification of Diseases, 9<sup>th</sup> revision, Clinical Modification; ICD-10CA: Enhanced version of International Classification of Diseases, 10<sup>th</sup> revision, for use in Canada; MADRS: Montgomery-Åsberg Depression Rating Scale; MDD: Major depressive disorder; MHI 5: Mental Health Inventory-5; MINI: Mini International Neuropsychiatric Interview; NR: Not reported; NS: Not significant; OGCT: Oral glucose challenge test; OR: Odds ratio; PHQ: Patient Health Questionnaire; PPD: Postpartum depression; RCT: Randomized controlled trial; SCID: Structured Clinical Interview for DSM-IV; SD: Standard deviation; VA: Veterans affairs; WHO-5: World Health Organization Well-Being Index.

depression and DIP, especially GDM. Two claims registry studies ( $n = 11024^{[46]}$ ;  $n = 839792^{[39]}$ ) reported that

women with DIP (except type 1 diabetes) were at increased risk of developing depression during or after



**Table 2 Outcomes of included studies involving women with pre-existing type 1 or type 2 diabetes**

First author study design	Definition/measures of depression	Timing of depression measures	Overall <i>n</i> Subgroups, <i>n</i>	Main outcomes/findings
Berger <sup>[25]</sup> Retrospective	EPDS $\geq 13$ or did not answer "No" to self-harm question	Within 4 d after delivery	Unselected, <i>n</i> = 322 History of mental illness, <i>n</i> = 215	Prevalence of pre-existing DM did not differ between women with or without postpartum depression in either the unselected group or the group with history of mental illness Of 5 women with pre-existing DM, none had depression
Callesen <sup>[30]</sup> Prospective, cohort	HADS $\geq 8$	8 wk gestation	<i>n</i> = 148 Type 1, <i>n</i> = 118 Type 2, <i>n</i> = 30	Women with DM and depression were more likely to have preterm delivery (54% <i>vs</i> 16%, <i>P</i> = 0.003) and less likely to be nulliparous (23% <i>vs</i> 54%, <i>P</i> = 0.03) than women with DM without depression
Dalfrå <sup>[34]</sup> Prospective	CES-D $\geq 16$	3 <sup>rd</sup> trimester and 8 wk after delivery	<i>n</i> = 245 Type 1, <i>n</i> = 30; No DM, <i>n</i> = 39	Mean (SD) CES-D scores at 3 <sup>rd</sup> trimester were 19.1 (9.6) among women with Type 1 DM and 18.0 (8.7) among women without DM ( <i>P</i> = 0.67) The severity of depressive symptoms increased from the 3 <sup>rd</sup> trimester to after delivery in women with Type 1 DM [estimated mean difference in CES-D score (95%CI): 6.6 (2.9-10.2)], but decreased in women without DM [-2.7 (-5.9-0.5), <i>P</i> < 0.0001 between groups]
Jovanovic <sup>[39]</sup> Retrospective, claims database	ICD-9 codes 311, 296.2, 296.3, 300.4, 301.12, 309.1	During pregnancy and/or within 3 mo after delivery	<i>n</i> = 839792 Type 1, <i>n</i> = 1125 Type 2, <i>n</i> = 10136 No DM, <i>n</i> = 773751	Prevalence of depression was 5.2% and 8.3% among women with type 1 and type 2 DM, respectively Prevalence of concurrent type 1 DM and depression was 0.006% Prevalence of concurrent type 2 DM and depression was 0.086% Relative risk (95%CI): of depression in women with type 1 DM <i>vs</i> women with no DM was 1.16 (0.86-1.56) Relative risk (95%CI): of depression in women with type 2 DM <i>vs</i> women with no DM was 1.84 (1.70-2.00)
Katon 2011 <sup>[41]</sup> Cross-sectional analysis of prospective cohort	PHQ-9	3 <sup>rd</sup> trimester	<i>n</i> = 2398 Pre-existing DM (type NR), <i>n</i> = 226; No DM, <i>n</i> = 1747	Prevalence (95%CI): of probable major depression among women with pre-existing DM was 5.8% (2.7%-8.8%) by PHQ-9 score, 8.9% (5.1%-12.6%) by antidepressant use, and 13.3% (8.8%-17.7%) by either PHQ-9 or antidepressant use, compared with the prevalence among women without DM [PHQ-9: 4.1% (3.2%-5.1%); antidepressants: 6.2% (5.1%-7.3%); PHQ-9 and antidepressants: 9.6% (8.2%-11.0%)] After adjusting for demographic characteristics, chronic medical conditions, and pregnancy variables, pre-existing DM was not associated with major or any antenatal depression ( <i>P</i> value not reported)
Katon 2014 (PPD) <sup>[42]</sup> Retrospective, hospital database	PHQ-9	2 <sup>nd</sup> or 3 <sup>rd</sup> trimester and 6 wk after delivery	<i>n</i> = 1423	Prevalence of pre-existing DM was higher in women with PPD (14.5%) than in women without PPD (6.9%) ( <i>P</i> = 0.02) Of 104 women with pre-existing DM, 12 (11.5%) had PPD Pre-existing DM was a risk factor for postpartum depression [OR (95%CI): 1.98 (1.12-3.52)] ( <i>P</i> = 0.02)
Kozhimannil <sup>[46]</sup> Retrospective cohort	ICD9 codes 296.2, 296.3, 300.4, 301.12, 309.1, and 311	During the 6 mo before and up to 1 yr after delivery	<i>n</i> = 11024 With pre-existing DM (type NR), <i>n</i> = 311 (taking insulin, <i>n</i> = 57); no DM, <i>n</i> = 10367	Prevalence of concurrent pre-existing DM and depression was 0.84% Prevalence of depression in women with pre-existing DM taking insulin was 14.0% <i>vs</i> 16.1% among women with pre-existing DM not taking insulin ( <i>P</i> value not reported)
Levy-Shiff <sup>[66]</sup> Prospective	BDI	2 <sup>nd</sup> trimester	<i>n</i> = 153 Pre-existing DM, <i>n</i> = 53 (type NR) No DM, <i>n</i> = 49	No significant difference in depression during 2 <sup>nd</sup> trimester between pre-existing DM [mean (SD) BDI score 6.17 (5.16)] and controls [6.59 (5.88)] ( <i>P</i> $\geq 0.05$ ) For sample as a whole, higher levels of cognitive assessment of pregnancy as a challenge was associated with lower depression ( <i>P</i> < 0.05) Among women with pre-existing DM, higher levels of medical support were associated with lower levels of depression ( <i>P</i> < 0.01)
Mei-Dan <sup>[50]</sup> Retrospective, health administration database	ICD-9, ICD-10CA, and/or DSM-IV (ICD codes NR)	Within 5 yr before pregnancy	<i>n</i> = 437941 With pre-pregnancy depression, <i>n</i> = 3724 No known mental illness, <i>n</i> = 432358	Prevalence of DM (type NR) within 1 year before the index pregnancy was significantly higher in women with pre-pregnancy depression (3.4%) than in women with no known mental illness (1.2%) ( <i>P</i> value NR) Prevalence of pre-existing DM and pre-pregnancy depression was 0.029%
Moore <sup>[67]</sup> Prospective	Depression Adjective Checklist; Perceived Stress Scale	3 <sup>rd</sup> trimester	<i>n</i> = 131 Pre-existing insulin-dependent DM, <i>n</i> = 73 High risk of preterm birth, <i>n</i> = 48 Low risk of preterm birth, <i>n</i> = 25	White women with DM who were tested at a private clinic had higher Depression Adjective Checklist and Perceived Stress Scale scores than any other group (variables of white <i>vs</i> black, private <i>vs</i> public medical centre, DM <i>vs</i> low or high risk of preterm birth) ( <i>P</i> value not reported)
Ragland <sup>[54]</sup> Prospective, cross-sectional	BDI > 13	During pregnancy	<i>n</i> = 50 Type 1 DM, <i>n</i> = 8 Type 2 DM, <i>n</i> = 20	Mean BDI score was 10.0 among women with type 1 DM and 17.1 among women with type 2 DM No women with type 1 DM and 12 (60%) women with type 2 DM had BDI > 13

<sup>1</sup> Räisänen 2013 <sup>[56]</sup>	ICD10 codes F31.3, F31.5, F32-34	Up to 6 wk postpartum or a history of depression	<i>n</i> = 511422	Prevalence of pre-existing DM: 8.4% of women without any depression ( <i>n</i> = 492103), 11.1% of women with history of depression but not PPD ( <i>n</i> = 17881), 14.6% of women with PPD but no history of depression ( <i>n</i> = 431), and 13.3% of women with both history of depression and PPD ( <i>n</i> = 1007) ( <i>P</i> ≤ 0.001)
<sup>1</sup> Räisänen 2014 <sup>[55]</sup>	ICD10 codes F31.3, F31.5, F32-34	At hospital discharge after delivery	<i>n</i> = 511938	Prevalence of pre-existing DM (type NR): 8.4% of women without any depression ( <i>n</i> = 493037), 10.9% of women with history of depression but not during pregnancy ( <i>n</i> = 14781), 11.6% of women with depression during pregnancy but no history of depression ( <i>n</i> = 2189), and 13.6% of women with both depression during pregnancy and history of depression ( <i>n</i> = 1931) ( <i>P</i> ≤ 0.001) Depression during pregnancy was not associated with pre-existing DM [adjusted OR (95%CI): = 1.10 (0.93-1.31)]
Singh <sup>[59]</sup>	BDI ≥ 10; self-reported medical history	During pregnancy	<i>n</i> = 152 History of depression, <i>n</i> = 39 No history of depression, <i>n</i> = 113	Type 2 DM was significantly more common in women with history of depression than in women with no history of depression ( <i>P</i> < 0.05) Of 39 women with history of depression, 5 (13%) had type 1 DM, and 19 (49%) had type 2 DM Of 113 women with no history of depression, 18 (16%) had type 1 DM, and 28 (25%) had type 2 DM
Sundaram <sup>[62]</sup>	Survey of PPD diagnosis; survey of symptoms based on PHQ-2	Postpartum	Up to 61733 pregnancies	In analysis of data from 22 states, pre-existing DM was not a significant predictor of PPD symptoms [OR (95%CI): 1.16 (0.78-1.59), <i>n</i> = 45669, <i>P</i> = 0.39] or diagnosis [OR (95%CI): 1.31 (0.45-3.06), <i>n</i> = 5924, <i>P</i> = 0.56] In analysis of data from 2 states that included both PPD symptoms and diagnosis on the survey, pre-existing DM was a significant predictor of PPD diagnosis [OR (95%CI): 5.65 (1.72-15.37), <i>n</i> = 2136, <i>P</i> < 0.01]

<sup>1</sup>The Räisänen *et al* 2013 and 2014 publications use the same database within the same time period (2002-2010) and, therefore, the study populations are almost identical. BDI: Beck Depression Inventory; CES-D: Center for Epidemiologic Studies Depression scale; CI: Confidence interval; DM: Diabetes mellitus; DSM-IV: Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; EPDS: Edinburgh Postnatal Depression Scale; HADS: Hospital Anxiety and Depression Scale; ICD: International Classification of Disease; ICD-10CA: enhanced version of International Classification of Diseases, 10<sup>th</sup> revision, for use in Canada; NR: Not reported; PHQ: Patient Health Questionnaire; PPD: Postpartum depression; SD: Standard deviation.

**Table 3 Outcomes of included studies involving women with any type of diabetes (not specified or data grouped)**

First author study design	Definition/measures of depression	Timing of depression measures	Overall <i>n</i> Subgroups, <i>n</i>	Main outcomes/findings
Ahmed <sup>[21]</sup> Prospective, cross-sectional	EPDS ≥ 10	6-8 wk postpartum	<i>n</i> = 1000 With DM (type NR), <i>n</i> = 31 No DM, <i>n</i> = 969	The proportion of women with DM who had PPD (51.6%) was significantly higher than the proportion of women without DM who had PPD (27.7%) ( <i>P</i> = 0.004) Calculated prevalence of women with both DM and PPD was 1.6% (16 of 1000)
Bansil <sup>[22]</sup> Retrospective	ICD9 codes 296.2, 296.3, 300.4, 311, 298.0, 309.0, 309.1	At the time of delivery	<i>n</i> = 32156438 With depression, <i>n</i> = 244939; With DM (type 1, type 2, or GDM), <i>n</i> = 1536514 With DM and depression, <i>n</i> = 18245	Rate of concurrent DM at the time of delivery higher in women with depression (74.5 per 1000 deliveries) <i>vs</i> women without depression (47.6 per 1000 deliveries; OR (95%CI): 1.52 (1.47-1.58)] Calculated prevalence of DM and depression = 0.06% (18245 of 32156438 deliveries)
Benute <sup>[24]</sup> Prospective	PRIME-MD	During prenatal outpatient visits/hospitalisation	<i>n</i> = 326 With DM, <i>n</i> = 84 With MDD, <i>n</i> = 29	Prevalence of DM in women with MDD was 7.1% Calculated prevalence of DM and MDD = 0.61% (7.1% of 29 = 2; 2/326 = 0.61%)
Berger <sup>[25]</sup> Retrospective	EPDS ≥ 13 or did not answer "No" to self-harm question	Within 4 d after delivery	Unselected, <i>n</i> = 322 History of mental illness, <i>n</i> = 215	Prevalence of any DM did not differ between women with or without postpartum depression in either the unselected group or the group with history of mental illness
Chen <sup>[32]</sup> Retrospective	ICD9 codes 296.2, 296.3, 300.4, and 311	History of depression within 2 years before delivery	<i>n</i> = 5283 With DM (type NR), <i>n</i> = 319	Calculated prevalence of DM among women with depression was 6.0%
Kozhimannil <sup>[46]</sup> Retrospective cohort	ICD9 codes 296.2, 296.3, 300.4, 301.12, 309.1, and 311	During the 6 mo before and up to 1 year after delivery	<i>n</i> = 11024 With DM (pre-existing or GDM), <i>n</i> = 657;	Overall calculated prevalence of women with both DM (any type) and depression was 1.1% Prevalence of depression among women with any DM was 15.2% <i>vs</i> 8.5% among women without DM ( <i>P</i> value not reported)
Ragland <sup>[54]</sup> Prospective, cross-sectional	BDI > 13	During pregnancy	No DM, <i>n</i> = 10367 <i>n</i> = 50 Type 1 DM, <i>n</i> = 8 Type 2 DM, <i>n</i> = 20 GDM, <i>n</i> = 22	Women with any DM had an increased odds of experiencing depression during or after pregnancy [OR (95%CI): 1.85 (1.45-2.36)] <i>vs</i> women without DM Women with any DM and no prenatal depression (9.6%) had increased odds of experiencing PPD or taking an antidepressant in the year after delivery [OR (95%CI): 1.69 (1.27-2.23)] <i>vs</i> women without DM

				Mean (SD) BDI score was 14.1 (9.9), range 3-43 Number (%) women with DM and severe (BDI $\geq$ 29), moderate (BDI 20-28), mild (BDI 14-19), and minimal (BDI 0-13) depression was 5 (10%), 8 (16%), 8 (16%), and 29 (58%) 42% of women with DM had BDI scores $>$ 13, indicating clinical depression Among patients with clinical depression, only 19% were receiving treatment for depression Number of pregnancies showed a positive correlation with BDI score ( $P = 0.0078$ ) Least mean squares of HbA1c level was higher, but not significantly, in women with depression [7.3% (56 mmol/mol)] than in those without [6.9% (52 mmol/mol)] ( $P \geq 0.05$ ) Calculated prevalence of DM (any type) and depression in pregnant women = 0.06%
Räisänen 2013 <sup>[56]</sup>	ICD10 codes F31.3, F31.5, and F32-34	Up to 6 wk postpartum or a history of depression	$n = 511422$	
Singh <sup>[59]</sup>	BDI $\geq$ 10; self-reported medical history	During pregnancy	$n = 152$	Current BDI scores were higher in women with DM and history of depression [mean (SD) 17.2 (11.5)] than in women with DM and no history of depression [7.8 (7.4), $P < 0.0001$ ] Percentage of women with BDI $\geq$ 10 significantly greater in women with DM and history of depression (72%) than in women with DM and no history of depression (28%, $P < 0.0001$ ) Most women did not report high levels of depression Among all women with DM, depression scores decreased significantly ( $P < 0.001$ ) over time [mean (SD) scores of 9.2 (6.6), 10.1 (8.3), 6.7 (8.2), 5.6 (7.0), and 3.8 (4.2) at 36 wk gestation, 2 d postpartum, 1 wk postpartum, 4 wk postpartum, and 8 wk postpartum, respectively] There were no differences between women with GDM and women with pre-existing DM in depression scores during pregnancy ( $P = 0.17$ ) or postpartum ( $P$ value not reported)
York <sup>[65]</sup>	Multiple Adjective Check List	36 wk gestation, and 2 d, 1 wk, 4 wk, and 8 wk postpartum	$n = 36$ Pre-existing DM, $n = 6$ GDM, $n = 30$	

BDI: Beck Depression Inventory; CI: Confidence interval; DM: Diabetes mellitus; EPDS: Edinburgh Postnatal Depression Scale; GDM: Gestational diabetes mellitus; ICD: International Classification of Disease; MDD: Major depressive disorder; NR: Not reported; PPD: Postpartum depression; PRIME-MD: Primary Care Evaluation of Mental Disorders classification system; SD: Standard deviation.

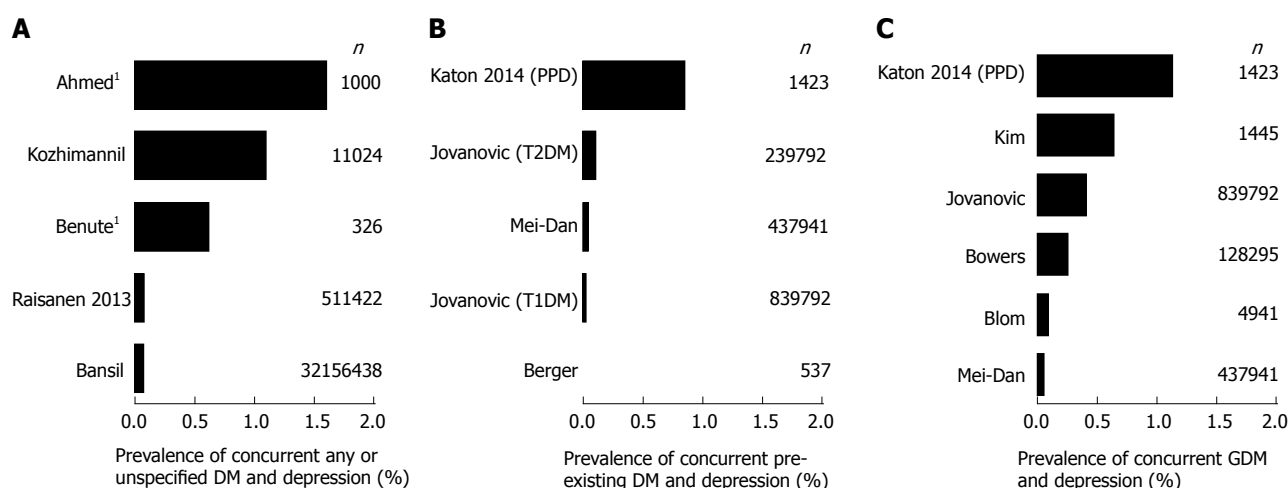
pregnancy relative to pregnant women without diabetes [any DIP: OR (95%CI): 1.85 (1.45-2.36)<sup>[46]</sup>; GDM: Relative risk (95%CI): 1.17 (1.12-1.21)<sup>[39]</sup>; type 2 diabetes: Relative risk (95%CI): 1.84 (1.70-2.00)<sup>[39]</sup>; type 1 diabetes: Relative risk (95%CI): 1.16 (0.86-1.56)<sup>[39]</sup>. Similarly, a maternal and infant database study ( $n = 1057647$ ) reported that GDM was significantly associated with increased risk of depression at the time of hospital discharge after delivery [adjusted OR (95%CI): 1.44 (1.26-1.65)]<sup>[64]</sup>. A hospital records review ( $n = 18192$  pregnancies) reported that GDM was significantly associated with increased risk of postpartum depression after adjustment for age, pre-eclampsia, and preterm birth [OR (95%CI): 1.46 (1.16-1.83);  $P = 0.001$ ], but not after adjustment for other clinical and socioeconomic factors [OR (95%CI): 1.29 (0.98-1.70);  $P = 0.064$ ]<sup>[63]</sup>. Conversely, another hospital records review ( $n = 128295$ ) reported that a history of depression was a risk factor for the development of GDM [OR (95%CI): 1.42 (1.26-1.60)]<sup>[28]</sup>. A national health database study ( $n > 32$  million) reported that women with depression at delivery were more likely to also have diabetes (type not specified) than women without depression [OR (95%CI): 1.52 (1.47-1.58)]<sup>[22]</sup>. In another national health database study that examined the relationship between reproductive risk factors and postpartum depression ( $n = 511422$ ), the prevalence of DIP (pre-existing or gestational) was greater among

women with a history of depression or with postpartum depression than among those without any depression<sup>[56]</sup>. This study also reported that in women with a history of depression, the risk of postpartum depression is increased in those who also have GDM [OR (95%CI): 1.62 (1.23-2.14)]. A related study using the same database reported that an increased prevalence of depression during pregnancy was associated with GDM [adjusted OR (95%CI): 1.29 (1.11-1.50)], but not with pre-existing diabetes [adjusted OR (95%CI): 1.10 (0.93-1.31)]<sup>[55]</sup>. The remaining health database studies that used ICD codes only reported prevalence data<sup>[32,40,50]</sup>.

The timing of depression assessment also varied (Tables 1-3). There were 22 studies that measured depression only during pregnancy<sup>[22,24,26,30,31,36,37,40,41,43,45,51,53-55,57-59,61,64,66,67]</sup>. Conversely, 11 studies focussed on postpartum depression, most commonly measured within the first 3 mo<sup>[20,21,23,25,27,29,33,47,52,62,63]</sup>. There were nine studies that measured depression during both pregnancy and postpartum<sup>[34,35,38,39,42,44,46,49,65]</sup> and five studies that classified participants based on a history of pre-pregnancy depression<sup>[28,32,50,56,60]</sup>.

### Prevalence of concurrent DIP and depression during or after pregnancy

The prevalence of concurrent DIP and depression in a general population sample of pregnant or post-



**Figure 2** Prevalence of concurrent diabetes and depression reported in studies included in this review. The *n* for each study represents the overall sample size. A: Prevalence of concurrent diabetes (types combined or not specified<sup>1</sup>) and depression; B: Prevalence of concurrent pre-existing diabetes and depression; C: Prevalence of concurrent gestational diabetes and depression. DM: Diabetes mellitus; GDM: Gestational diabetes mellitus; PPD: Postpartum depression; T1DM: Type 1 diabetes mellitus; T2DM: Type 2 diabetes mellitus.

partum women was reported or could be calculated from data in 12 retrospective or cross-sectional studies<sup>[21,22,24,25,27,28,39,42,44,46,50,56]</sup> and ranged from 0% to 1.6% (median 0.61%) (Figure 2). The prevalence of depression during or after pregnancy concurrent with any or unspecified diabetes ranged from 0.06% to 1.6% (5 studies; median 0.61%) (Figure 2A). The prevalence of concurrent pre-existing diabetes and depression during or after pregnancy ranged from 0.006% (type 1 diabetes only) to 1.1% (4 studies, median 0.03%) (Figure 2B). The prevalence of concurrent GDM and depression during or after pregnancy ranged from 0.029% to 1.12% (6 studies, median 0.32%) (Figure 2C).

### Gestational diabetes

Among women with GDM (Table 1, Figure 3), the reported prevalence of depression during or after pregnancy ranged widely, from 4.1% to 80% (16 studies<sup>[26,31,33,35,39-41,43,44,46,51,52,54,57,58,61]</sup>, median 14.7%). Heterogeneity in sample size, the definition of depression, and the timing of its assessment is likely to have contributed to this wide range of prevalence rates.

The prevalence of GDM among women with a history of depression, reported in seven studies<sup>[23,27,28,48,50,55,56]</sup>, ranged from 1.0%<sup>[27]</sup> to 17.6% (women with both history of depression and postpartum depression<sup>[55,56]</sup>) (Table 1).

### Pre-existing diabetes

Among women with pre-existing diabetes (Table 2), the prevalence of depression during or after pregnancy ranged from 0% to 60% (6 studies, median 8.3%), similar to the broad range reported for women with GDM. The prevalence of depression during or after pregnancy in women with pre-existing diabetes was 0% (in a small sample of five women with pre-existing diabetes)<sup>[25]</sup>, 0% (in a small sample of eight women with type 1 diabetes)<sup>[54]</sup>, 5.2% (type 1 diabetes)<sup>[39]</sup>,

5.8%<sup>[41]</sup>, 8.3% (type 2 diabetes)<sup>[39]</sup>, 11.5%<sup>[42]</sup>, 14.0% (women taking insulin)<sup>[46]</sup>, 16.1% (women not taking insulin)<sup>[46]</sup>, and 60% (women with type 2 diabetes)<sup>[54]</sup>.

### Diabetes as a risk factor for depression during or after pregnancy

Many of the studies examined whether DIP was a risk factor for depression during or after pregnancy, or compared the prevalence of depression between women with DIP and pregnant women without diabetes. Overall, there was no consensus regarding whether women with DIP were more likely to have depression than pregnant women without diabetes.

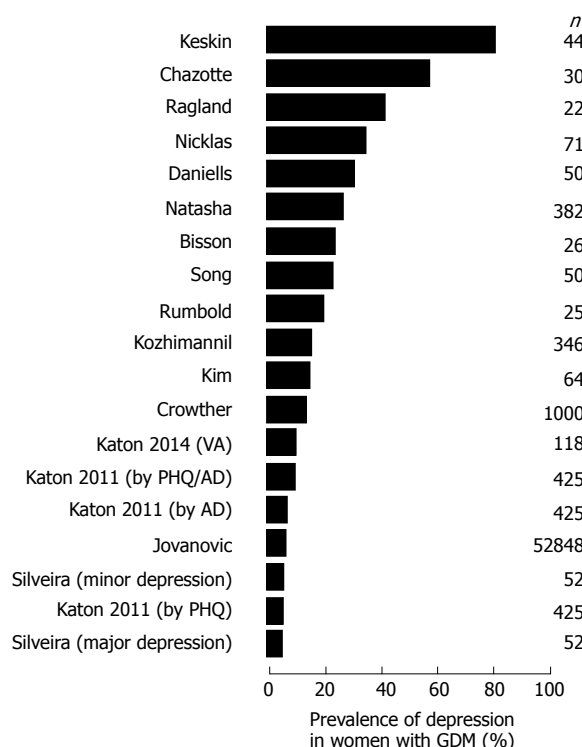
### Gestational diabetes

In 11 studies<sup>[20,25,26,29,35,39,51,55,56,61,64]</sup>, women with GDM had a significantly greater prevalence or risk of depression during or after pregnancy than pregnant women without diabetes (Table 1). In two of these studies, a significant effect of GDM was observed only for one subgroup of women (Qatari women, but not other Arab women<sup>[29]</sup>; women with a history of depression, but not women without a history of depression<sup>[56]</sup>). In one study<sup>[35]</sup>, the prevalence of depression among women with GDM was significantly greater than pregnant women without diabetes at 30 wk gestation, but not at 36 wk gestation or postpartum. In contrast, 16 studies reported no significant effect of GDM on the prevalence or risk of depression<sup>[23,27,31,34,38,41-44,47,49,57,58,62,63,66]</sup>.

### Pre-existing diabetes

Four studies reported no significant difference in depression between pregnant women with pre-existing diabetes and those without diabetes<sup>[25,41,55,66]</sup> (Table 2). One exploratory study was inconclusive, reporting that pre-existing diabetes was a significant predictor of postpartum depression diagnosis in a subset of data from two states of the United States, but not in the





**Figure 3** Prevalence of depression reported among women with gestational diabetes in studies included in this review. The *n* for each study represents the sample size of women with gestational diabetes. AD: Antidepressant medication; GDM: Gestational diabetes mellitus; PHQ: Patient Health Questionnaire; VA: Veterans Affairs.

nationwide analysis set<sup>[62]</sup>. In one retrospective study, pre-existing type 2 diabetes, but not type 1 diabetes, was associated with an increased risk of depression during or after pregnancy<sup>[39]</sup>. In another retrospective study, pre-existing diabetes was identified as a risk factor for postpartum depression<sup>[42]</sup>.

### Any type of diabetes

Two studies reported a greater prevalence<sup>[21]</sup> or risk (OR)<sup>[46]</sup> of depression among women with any type of DIP compared with pregnant women without diabetes (Table 3). One study reported a significant increase in the severity of depressive symptoms between the third trimester and postpartum among women with GDM or type 1 diabetes, but not among pregnant women without diabetes<sup>[34]</sup>. Another study reported no difference in the prevalence of any diabetes between women with postpartum depression and those without postpartum depression<sup>[25]</sup>.

### Depression as a risk factor for gestational diabetes

Several studies examined whether depression was a risk factor for the development of GDM, but again, there was no consensus (Table 1). Two studies of the same national database reported a greater prevalence<sup>[55,56]</sup> and one study reported a greater risk (OR<sup>[28]</sup>) of GDM among women with a pre-pregnancy history of depression. In contrast, two studies reported no difference in the prevalence of GDM (or abnormal glucose levels) among

women with depression early in pregnancy compared with women without depression<sup>[37,53]</sup>, and a third study reported similar prevalence rates of GDM in women with and without pre-pregnancy depression<sup>[50]</sup>.

### Treatment and management

Our literature search did not identify any specific guidelines on the treatment or management of women with both DIP and depression during or after pregnancy. Very few studies reported on the effects of treatment of either diabetes or depression on outcomes. In the completed RCT<sup>[33]</sup>, a significantly lower proportion of women with GDM who received dietary advice, performed blood glucose monitoring, and were treated with insulin therapy as needed had postpartum depression compared with women with GDM who received usual obstetric care (8% vs 17%;  $P = 0.001$ ). In the prospective study by Dalfrà *et al.*<sup>[34]</sup>, mean depressive symptom scores during the third trimester did not differ between women with GDM who were managed with diet only and women with GDM who were treated with insulin ( $P = 0.58$ ). In the retrospective study by Kozhimannil *et al.*<sup>[46]</sup>, the prevalence of depression during or after pregnancy among women with GDM who were treated with insulin was slightly higher than in women who were not treated with insulin (16.0% vs 13.7%;  $P$  value not reported). In the same study, the prevalence of depression among women with pre-existing diabetes was slightly lower in those who were treated with insulin than in those who were not (14.0% vs 16.1%;  $P$  value not reported). In the prospective study by Levy-Shiff *et al.*<sup>[66]</sup>, higher levels of patient-reported support from medical staff were associated with lower levels of depression in women with pre-existing diabetes ( $P < 0.01$ ). Similarly, in the prospective study by Ko *et al.*<sup>[45]</sup>, women with GDM who participated in a 4-week educational coaching program had a greater decrease in depression scores than those who did not participate. In the prospective study by Ragland *et al.*<sup>[54]</sup>, only 19% of women with concurrent DIP (any type) and depression (Beck Depression Inventory score  $> 13$ ) were receiving treatment for depression. In the same study, the HbA1c level was numerically higher, but not significantly higher, in women with both DIP and depression compared with pregnant women without depression [7.3% (56 mmol/mol) vs 6.9% (52 mmol/mol);  $P \geq 0.05$ ].

## DISCUSSION

This is the first systematic literature review assessing what is known about women who have both DIP and depression during pregnancy or postpartum. Despite the number of studies identified, there was no clear consensus on whether women with DIP are more likely to develop depression than pregnant women without diabetes, or whether women with depression were more likely to develop GDM. Heterogeneity in the definition of depression, the scales used to measure depressive symptoms, the timing of measures, and the types of diabetes examined, together with the poor quality and

observational nature of most of the studies, are likely to have contributed to the lack of consensus. Further, the primary objective of many studies was not directly relevant to this review and the results we report were often secondary or incidental findings. Importantly, we did not identify any guidelines for the management of women with both DIP and depression. Given that 0.006% to 1.6% (median 0.61%) of pregnant women are reported to have both diabetes and depression, and that this prevalence is likely to rise, guidance on managing these women would be valuable to healthcare professionals.

Although many of the studies in this review examined the relationship between DIP and depression, there was no consensus on whether women with DIP are at greater risk of depression than pregnant women without diabetes. The reasons for the disparate results among the studies may in part be due to different definitions of depression and the timing of its measurement, as well as differences in study population, outcomes, and objectives. Only a quarter of the studies used a diagnosis of depression instead of symptoms, which may have made it more difficult to establish if there was a link. For example, in a meta-analysis of studies involving non-pregnant patients, diabetes was identified as a significant risk factor for depression as defined by diagnosis or prescription of antidepressants, but not when depression was defined by symptoms using questionnaires<sup>[9]</sup>. However, almost all the large, retrospective database studies that used ICD codes to define depression were suggestive of an increased prevalence or risk of depression among women with DIP, especially those with GDM<sup>[22,39,46,55,56,64]</sup>.

Although the exact mechanisms that link diabetes and depression are not known, especially in pregnant or postpartum women, current hypotheses in non-pregnant patients focus on both psychological and biological factors<sup>[13]</sup>. For example, the higher prevalence of depression in patients with diabetes may be related to the burden of coping with a chronic disease<sup>[69]</sup>. Conversely, depression is often associated with lifestyle choices, such as poor diet and lack of exercise, which may increase the risk of developing type 2 diabetes. However, these behavioral factors do not account for all of the increased risk of diabetes in patients with depression<sup>[70,71]</sup>. Depression and diabetes may also share some biological pathologies, such as altered activity of the hypothalamic-pituitary-adrenal axis, sympathetic nervous system, and inflammatory processes<sup>[72]</sup>. Regardless of the underlying mechanisms, there is now considerable evidence that diabetes and depression are closely linked and that patients with either disease are at increased risk of developing the other<sup>[8-12]</sup>. Whether the same mechanisms are involved in linking depression with diabetes in pregnancy remains unclear, and studies designed to investigate these mechanisms are required.

Few studies examined the potential role of treatment or glycemic control on depression in women with DIP. Among these, the RCT by Crowther *et al.*<sup>[33]</sup> reported

that women with GDM who received active intervention (dietary advice, glucose monitoring, and insulin therapy, if needed) were significantly less likely to develop postpartum depression than women receiving routine obstetric care. Unfortunately, measures of glycemic control and their relationship to postpartum depression were not reported. A previous meta-analysis has indicated that depression among non-pregnant patients with diabetes was significantly associated with poorer glycemic control<sup>[73]</sup>. However, there is no similar evidence for a relationship between glycemic control and depression among pregnant women.

There was also no consensus among the few studies that examined whether pre-pregnancy depression increased the risk of GDM. Given that depression is linked to obesity and insulin resistance<sup>[13]</sup>, women with depression who become pregnant should be carefully monitored for impaired glucose tolerance. In addition, certain antidepressant and centrally acting antipsychotic medications may increase the risk of type 2 diabetes<sup>[74]</sup>. This relationship is attributable to several mechanisms, both associated with and independent of weight gain<sup>[74]</sup>, and a similar relationship may exist for GDM.

This review is strengthened by the systematic methods used to identify publications and by the absence of restrictions on publication date or language. In addition, the inclusion of studies involving all types of diabetes and definitions of depression increased the number of publications reviewed. However, the resulting heterogeneity, especially in the definition of depression, is likely to have contributed to the lack of consensus. Indeed, our original intent was to only include studies that used a formal clinical diagnosis of depression. However, preliminary searches revealed that few such studies exist and most of those that do are retrospective. For this reason, we expanded our inclusion criteria to also capture studies that used measures of depressive symptoms, allowing us to assess the wider body of evidence on this topic.

Our review is also limited by the observational nature of almost all the studies and because many of the studies were not designed to examine the relationship between depression and DIP. Observational studies are subject to a range of potential biases, including selection bias, information bias, recall bias, and attrition bias. In addition, many of the articles included in the review were poorly reported, making assessment of the true quality of individual studies difficult. Most studies did not report outcomes of specific interest to us, such as the effect of treatment for depression or diabetes on maternal outcomes, risk factors that contribute to co-occurrence of depression and DIP, and prevalence rates, many of which we calculated from reported data. However, RCTs involving pregnant women are uncommon because of ethical considerations, and observational studies may be the only way to examine the relationship between depression and DIP.

Importantly, we did not identify any specific guidelines for the management of women with both DIP and

depression during or after pregnancy. Unfortunately, major clinical treatment guidelines for diabetes and depression do not address these patients. The American Diabetes Association (ADA) Standards of Medical Care recommend routine screening for depression in patients with diabetes, but any special care for pregnant women is not addressed<sup>[15]</sup>. Similarly, the American College of Obstetricians and Gynecologists Practice Bulletin on GDM does not address mental health issues<sup>[16]</sup>. Although the American Psychiatric Association Practice Guideline for major depressive disorder provides guidance for patients who also have diabetes or are pregnant, it does not provide guidance for women who have DIP<sup>[18]</sup>. However, limited management guidance for women with DIP and depression is provided by some country-specific guidelines (e.g., Germany<sup>[75]</sup> and India<sup>[76]</sup>). In addition, a consensus statement published by the ADA in 2008 recommends screening for depression before and during pregnancy in women with pre-existing diabetes<sup>[77]</sup>. Although the consensus statement indicates that the management plan should be adjusted in women with DIP and depression, the only recommendation provided is to use structured psychotherapy as first-line treatment for mild depression<sup>[77]</sup>. Given the expected increase in the number of women with DIP and depression, together with the particular challenges these women face in caring for themselves and their children, healthcare professionals need more specific guidance on management strategies for these patients. A collaborative care approach involving primary care physicians and specialists improves outcomes in non-pregnant patients with both diabetes and depression<sup>[78]</sup>, and a similar model may be effective for the management of pregnant and postpartum women. Such guidance, however, should be based on sound research evidence, which, as our review demonstrates, is currently lacking. In agreement with the results of our systematic review, two narrative reviews<sup>[5,6]</sup> and a systematic review focussing on the transition to motherhood in women with type 1 diabetes<sup>[79]</sup> have recognized that rigorous research into DIP and depression (and other psychosocial issues) is much needed. In addition, greater awareness of depression is needed among clinicians who treat women with diabetes, which will allow for better planning and management of pregnancy.

In conclusion, this systematic review highlights the need for additional, high-quality research into the relationship between DIP and depression. Such research is needed to inform the development of evidence-based guidelines that will help clinicians care for women with both DIP and depression.

## ACKNOWLEDGMENTS

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(GPP3).

## COMMENTS

### Background

Diabetes in pregnancy (DIP) has adverse effects on women and their children, as does depression during pregnancy or postpartum. Both DIP and depression are increasingly common, and it is likely that the number of women with both conditions is also growing. However, major diabetes and mental health guidelines do not provide adequate advice regarding care of patients with both DIP and depression.

### Research frontiers

At present, the prevalence of women with concurrent DIP and depression has not been established. In addition, recent evidence suggests a bidirectional relationship between diabetes and depression among non-pregnant patients, but it is not known if a similar link exists in pregnant or postpartum patients.

### Innovations and breakthroughs

This is the first systematic literature review assessing what is known about women who have both DIP and depression during pregnancy or postpartum. Despite the number of studies identified ( $n = 48$ ), there was no clear consensus on whether women with DIP are more likely to develop depression than pregnant women without diabetes, or whether women with depression were more likely to develop gestational diabetes. Importantly, they did not identify any guidelines for the management of women with both DIP and depression.

### Applications

This systematic review highlights the need for additional, high-quality research into the relationship between DIP and depression. Such research is needed to inform the development of evidence-based guidelines that will help clinicians care for women with both DIP and depression.

### Terminology

Women with DIP include those who had pre-existing type 1 or type 2 diabetes mellitus before becoming pregnant and those who developed gestational diabetes mellitus during pregnancy. Gestational diabetes mellitus is characterized by elevated blood glucose levels that develop during mid-pregnancy and that usually resolve after childbirth.

### Peer-review

This manuscript is a systematic review of the literature about the relationship between depression (postpartum depression in particular) and diabetes in pregnancy. The assessment of the articles indicated overall poor study quality as many studies were observational and often lacked stringent, objective criteria to support a diagnosis of clinical depression. The main conclusion of the authors is that high quality research with stringent criteria and assessable parameters is needed to establish specific guidelines for management of pregnant women with depression and diabetes.

## REFERENCES

- 1 **International Diabetes Federation.** IDF Diabetes Atlas. 7th ed. Available from: URL: <http://www.idf.org/diabetesatlas>
- 2 **Veeraswamy S, Vijayam B, Gupta VK, Kapur A.** Gestational diabetes: the public health relevance and approach. *Diabetes Res Clin Pract* 2012; **97**: 350-358 [PMID: 22726771 DOI: 10.1016/j.diabetes.2012.04.024]
- 3 **Bardenheier BH, Imperatore G, Devlin HM, Kim SY, Cho P, Geiss LS.** Trends in pre-pregnancy diabetes among deliveries in 19 U.S. states, 2000-2010. *Am J Prev Med* 2015; **48**: 154-161 [PMID: 25326417 DOI: 10.1016/j.amepre.2014.08.031]
- 4 **Bardenheier BH, Imperatore G, Gilboa SM, Geiss LS, Saydah SH, Devlin HM, Kim SY, Gregg EW.** Trends in Gestational Diabetes Among Hospital Deliveries in 19 U.S. States, 2000-2010. *Am J Prev Med* 2015; **49**: 12-19 [PMID: 26094225 DOI: 10.1016/

- j.amepre.2015.01.026]
- 5 **Byrn MA**, Penckofer S. Antenatal depression and gestational diabetes: a review of maternal and fetal outcomes. *Nurs Womens Health* 2013; **17**: 22-33 [PMID: 23399010 DOI: 10.1111/1751-486X.12003]
- 6 **Rasmussen-Torvik LJ**, Harlow BL. The association between depression and diabetes in the perinatal period. *Curr Diab Rep* 2010; **10**: 217-223 [PMID: 20425585 DOI: 10.1007/s11892-010-0108-4]
- 7 **DelRosario GA**, Chang AC, Lee ED. Postpartum depression: symptoms, diagnosis, and treatment approaches. *JAAPA* 2013; **26**: 50-54 [PMID: 23409386 DOI: 10.1097/01720610-201302000-00009]
- 8 **Nouwen A**, Winkley K, Twisk J, Lloyd CE, Peyrot M, Ismail K, Pouwer F. Type 2 diabetes mellitus as a risk factor for the onset of depression: a systematic review and meta-analysis. *Diabetologia* 2010; **53**: 2480-2486 [PMID: 20711716 DOI: 10.1007/s00125-010-1874-x]
- 9 **Rotella F**, Mannucci E. Diabetes mellitus as a risk factor for depression. A meta-analysis of longitudinal studies. *Diabetes Res Clin Pract* 2013; **99**: 98-104 [PMID: 23265924 DOI: 10.1016/j.diabres.2012.11.022]
- 10 **Roy T**, Lloyd CE. Epidemiology of depression and diabetes: a systematic review. *J Affect Disord* 2012; **142** Suppl: S8-21 [PMID: 23062861 DOI: 10.1016/S0165-0327(12)70004-6]
- 11 **Knol MJ**, Twisk JW, Beekman AT, Heine RJ, Snoek FJ, Pouwer F. Depression as a risk factor for the onset of type 2 diabetes mellitus. A meta-analysis. *Diabetologia* 2006; **49**: 837-845 [PMID: 16520921 DOI: 10.1007/s00125-006-0159-x]
- 12 **Rotella F**, Mannucci E. Depression as a risk factor for diabetes: a meta-analysis of longitudinal studies. *J Clin Psychiatry* 2013; **74**: 31-37 [PMID: 23419223 DOI: 10.4088/JCP.12r07922]
- 13 **Oladeji BD**, Gureje O. The comorbidity between depression and diabetes. *Curr Psychiatry Rep* 2013; **15**: 390 [PMID: 23933977 DOI: 10.1007/s11920-013-0390-3]
- 14 **Gonzalez JS**, Peyrot M, McCarl LA, Collins EM, Serpa L, Mimiaga MJ, Safren SA. Depression and diabetes treatment nonadherence: a meta-analysis. *Diabetes Care* 2008; **31**: 2398-2403 [PMID: 19033420 DOI: 10.2337/dc08-1341]
- 15 Introduction. *Diabetes Care* 2016; **39** Suppl 1: S1-S2 [PMID: 26696671 DOI: 10.2337/dc16-S001]
- 16 Practice Bulletin No. 137: Gestational diabetes mellitus. *Obstet Gynecol* 2013; **122**: 406-416 [PMID: 23969827 DOI: 10.1097/01.AOG.0000433006.09219.f1]
- 17 **NICE**. NICE Clinical Guideline 3. Diabetes in pregnancy: management from preconception to the postnatal period. Available from: URL: <http://www.nice.org.uk/guidance/cg3>
- 18 **American Psychiatric Association**. Practice guideline for the treatment of patients with major depressive disorder. 3rd ed. Available from: URL: [http://psychiatryonline.org/pb/assets/raw/sitewide/practice\\_guidelines/guidelines/mdd.pdf](http://psychiatryonline.org/pb/assets/raw/sitewide/practice_guidelines/guidelines/mdd.pdf)
- 19 **NICE**. NICE Clinical Guideline 192. Antenatal and postnatal mental health: clinical management and service guidance. Available from: URL: <https://www.nice.org.uk/guidance/cg192>
- 20 **Abdollahi F**, Zarghami M, Azhar MZ, Sazlina SG, Lye MS. Predictors and incidence of post-partum depression: a longitudinal cohort study. *J Obstet Gynaecol Res* 2014; **40**: 2191-2200 [PMID: 25132641 DOI: 10.1111/jog.12471]
- 21 **Ahmed HM**, Alalaf SK, Al-Tawil NG. Screening for postpartum depression using Kurdish version of Edinburgh postnatal depression scale. *Arch Gynecol Obstet* 2012; **285**: 1249-1255 [PMID: 22159747 DOI: 10.1007/s00404-011-2165-6]
- 22 **Bansil P**, Kuklina EV, Meikle SF, Posner SF, Kourtis AP, Ellington SR, Jamieson DJ. Maternal and fetal outcomes among women with depression. *J Womens Health (Larchmt)* 2010; **19**: 329-334 [PMID: 20095906 DOI: 10.1089/jwh.2009.1387]
- 23 **Bener A**, Burgut FT, Ghuloum S, Sheikh J. A study of postpartum depression in a fast developing country: prevalence and related factors. *Int J Psychiatry Med* 2012; **43**: 325-337 [PMID: 23094465 DOI: 10.2190/PM.43.4.c]
- 24 **Benute GR**, Nomura RM, Reis JS, Fraguas Junior R, Lucia MC, Zugaib M. Depression during pregnancy in women with a medical disorder: risk factors and perinatal outcomes. *Clinics (Sao Paulo)* 2010; **65**: 1127-1131 [PMID: 21243285 DOI: 10.1590/S1807-59322010001100013]
- 25 **Berger E**, Wu A, Smulian EA, Quiñones JN, Curet S, Marraccini RL, Smulian JC. Universal versus risk factor-targeted early inpatient postpartum depression screening. *J Matern Fetal Neonatal Med* 2015; **28**: 739-744 [PMID: 24987874 DOI: 10.3109/14767058.2014.932764]
- 26 **Bisson M**, Sériès F, Giguère Y, Pamidi S, Kimoff J, Weisnagel SJ, Marc I. Gestational diabetes mellitus and sleep-disordered breathing. *Obstet Gynecol* 2014; **123**: 634-641 [PMID: 24499765 DOI: 10.1097/AOG.0000000000000143]
- 27 **Blom EA**, Jansen PW, Verhulst FC, Hofman A, Raat H, Jaddoe VW, Coolman M, Steegers EA, Tiemeier H. Perinatal complications increase the risk of postpartum depression. The Generation R Study. *BJOG* 2010; **117**: 1390-1398 [PMID: 20682022 DOI: 10.1111/j.1471-0528.2010.02660.x]
- 28 **Bowers K**, Laughon SK, Kim S, Mumford SL, Brite J, Kiely M, Zhang C. The association between a medical history of depression and gestational diabetes in a large multi-ethnic cohort in the United States. *Paediatr Perinat Epidemiol* 2013; **27**: 323-328 [PMID: 23772933 DOI: 10.1111/ppe.12057]
- 29 **Burgut FT**, Bener A, Ghuloum S, Sheikh J. A study of postpartum depression and maternal risk factors in Qatar. *J Psychosom Obstet Gynaecol* 2013; **34**: 90-97 [PMID: 23701432 DOI: 10.3109/0167482X.2013.786036]
- 30 **Callesen NF**, Secher AL, Cramon P, Ringholm L, Watt T, Damm P, Mathiesen ER. Mental health in early pregnancy is associated with pregnancy outcome in women with pregestational diabetes. *Diabet Med* 2015; **32**: 1484-1491 [PMID: 25864857 DOI: 10.1111/dme.12777]
- 31 **Chazotte C**, Freda MC, Elovitz M, Yousah J. Maternal depressive symptoms and maternal-fetal attachment in gestational diabetes. *J Womens Health* 1995; **4**: 375-380 [DOI: 10.1089/jwh.1995.4.375]
- 32 **Chen CH**, Lin HC. Prenatal care and adverse pregnancy outcomes among women with depression: a nationwide population-based study. *Can J Psychiatry* 2011; **56**: 273-280 [PMID: 21586193]
- 33 **Crowther CA**, Hiller JE, Moss JR, McPhee AJ, Jeffries WS, Robinson JS. Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. *N Engl J Med* 2005; **352**: 2477-2486 [PMID: 15951574 DOI: 10.1056/NEJMoa042973]
- 34 **Dalfrà MG**, Nicolucci A, Bisson T, Bonsembiante B, Lapolla A. Quality of life in pregnancy and post-partum: a study in diabetic patients. *Qual Life Res* 2012; **21**: 291-298 [PMID: 21633879 DOI: 10.1007/s11136-011-9940-5]
- 35 **Daniells S**, Grenyer BF, Davis WS, Coleman KJ, Burgess JA, Moses RG. Gestational diabetes mellitus: is a diagnosis associated with an increase in maternal anxiety and stress in the short and intermediate term? *Diabetes Care* 2003; **26**: 385-389 [PMID: 12547867 DOI: 10.2337/diacare.26.2.385]
- 36 **de Wit L**, Jelsma JG, van Poppel MN, Bogaerts A, Simmons D, Desoye G, Corcoy R, Kautzky-Willer A, Harreiter J, van Assche A, Devlieger R, Timmerman D, Hill D, Damm P, Mathiesen ER, Wender-Ozegowska E, Zawiejska A, Rebollo P, Lapolla A, Dalfrà MG, Del Prato S, Bertolotto A, Dunne F, Jensen DM, Andersen L, Snoek FJ. Physical activity, depressed mood and pregnancy worries in European obese pregnant women: results from the DALI study. *BMC Pregnancy Childbirth* 2015; **15**: 158 [PMID: 26228253 DOI: 10.1186/s12884-015-0595-z]
- 37 **Ertel KA**, Silveira M, Pekow P, Braun B, Manson JE, Solomon CG, Markenson G, Chasan-Taber L. Prenatal depressive symptoms and abnormalities of glucose tolerance during pregnancy among Hispanic women. *Arch Womens Ment Health* 2014; **17**: 65-72 [PMID: 24057869 DOI: 10.1007/s00737-013-0379-2]
- 38 **Huang T**, Rifas-Shiman SL, Ertel KA, Rich-Edwards J, Kleinman K, Gillman MW, Oken E, James-Todd T. Pregnancy Hyperglycaemia and Risk of Prenatal and Postpartum Depressive Symptoms. *Paediatr Perinat Epidemiol* 2015; **29**: 281-289 [PMID: 26058318 DOI: 10.1111/ppe.12199]
- 39 **Jovanović L**, Liang Y, Weng W, Hamilton M, Chen L, Wintfeld



- N. Trends in the incidence of diabetes, its clinical sequelae, and associated costs in pregnancy. *Diabetes Metab Res Rev* 2015; **31**: 707-716 [PMID: 25899622 DOI: 10.1002/dmrr.2656]
- 40 **Katon J**, Mattocks K, Zephyrin L, Reiber G, Yano EM, Callegari L, Schwarz EB, Goulet J, Shaw J, Brandt C, Haskell S. Gestational diabetes and hypertensive disorders of pregnancy among women veterans deployed in service of operations in Afghanistan and Iraq. *J Womens Health (Larchmt)* 2014; **23**: 792-800 [PMID: 25090022 DOI: 10.1089/jwh.2013.4681]
- 41 **Katon JG**, Russo J, Gavin AR, Melville JL, Katon WJ. Diabetes and depression in pregnancy: is there an association? *J Womens Health (Larchmt)* 2011; **20**: 983-989 [PMID: 21668382 DOI: 10.1089/jwh.2010.2662]
- 42 **Katon W**, Russo J, Gavin A. Predictors of postpartum depression. *J Womens Health (Larchmt)* 2014; **23**: 753-759 [PMID: 25121562 DOI: 10.1089/jwh.2014.4824]
- 43 **Keskin FE**, Ozyazar M, Pala AS, Elmali AD, Yilmaz B, Uygungolu U, Bozulolcay M, Tuten A, Bingöl A, Hatipoglu E. Evaluation of cognitive functions in gestational diabetes mellitus. *Exp Clin Endocrinol Diabetes* 2015; **123**: 246-251 [PMID: 25868060 DOI: 10.1055/s-0034-1395634]
- 44 **Kim C**, Brawarsky P, Jackson RA, Fuentes-Afflick E, Haas JS. Changes in health status experienced by women with gestational diabetes and pregnancy-induced hypertensive disorders. *J Womens Health (Larchmt)* 2005; **14**: 729-736 [PMID: 16232105 DOI: 10.1089/jwh.2005.14.729]
- 45 **Ko JM**, Lee JK. [Effects of a coaching program on comprehensive lifestyle modification for women with gestational diabetes mellitus]. *J Korean Acad Nurs* 2014; **44**: 672-681 [PMID: 25608545 DOI: 10.4040/jkan.2014.44.6.672]
- 46 **Kozhimannil KB**, Pereira MA, Harlow BL. Association between diabetes and perinatal depression among low-income mothers. *JAMA* 2009; **301**: 842-847 [PMID: 19244191 DOI: 10.1001/jama.2009.201]
- 47 **Liu CH**, Tronick E. Rates and predictors of postpartum depression by race and ethnicity: results from the 2004 to 2007 New York City PRAMS survey (Pregnancy Risk Assessment Monitoring System). *Matern Child Health J* 2013; **17**: 1599-1610 [PMID: 23095945 DOI: 10.1007/s10995-012-1171-z]
- 48 **Manoudi F**, Chagh R, Benhima I, Asri F, Diouri A, Tazi I. [Depressive disorders in diabetic patients]. *Encephale* 2012; **38**: 404-410 [PMID: 23062454 DOI: 10.1016/j.encep.2012.01.010]
- 49 **Mautner E**, Greimel E, Trutnovsky G, Daghofer F, Egger JW, Lang U. Quality of life outcomes in pregnancy and postpartum complicated by hypertensive disorders, gestational diabetes, and preterm birth. *J Psychosom Obstet Gynaecol* 2009; **30**: 231-237 [PMID: 19845493 DOI: 10.3109/01674820903254757]
- 50 **Mei-Dan E**, Ray JG, Vigod SN. Perinatal outcomes among women with bipolar disorder: a population-based cohort study. *Am J Obstet Gynecol* 2015; **212**: 367.e1-367.e8 [PMID: 25446660 DOI: 10.1016/j.ajog.2014.10.020]
- 51 **Natasha K**, Hussain A, Khan AK. Prevalence of depression among subjects with and without gestational diabetes mellitus in Bangladesh: a hospital based study. *J Diabetes Metab Disord* 2015; **14**: 64 [PMID: 26221580 DOI: 10.1186/s40200-015-0189-3]
- 52 **Nicklas JM**, Miller LJ, Zera CA, Davis RB, Levkoff SE, Seely EW. Factors associated with depressive symptoms in the early postpartum period among women with recent gestational diabetes mellitus. *Matern Child Health J* 2013; **17**: 1665-1672 [PMID: 23124798 DOI: 10.1007/s10995-012-1180-y]
- 53 **O'Brien LM**, Owusu JT, Swanson LM. Habitual snoring and depressive symptoms during pregnancy. *BMC Pregnancy Childbirth* 2013; **13**: 113 [PMID: 23679132 DOI: 10.1186/1471-2393-13-113]
- 54 **Ragland D**, Payakachat N, Hays EB, Banken J, Dajani NK, Ott RE. Depression and diabetes: Establishing the pharmacist's role in detecting comorbidity in pregnant women. *J Am Pharm Assoc* (2003) 2010; **50**: 195-199 [PMID: 20199962 DOI: 10.1331/JAPH A.2010.09191]
- 55 **Räisänen S**, Lehto SM, Nielsen HS, Gissler M, Kramer MR, Heinonen S. Risk factors for and perinatal outcomes of major depression during pregnancy: a population-based analysis during 2002-2010 in Finland. *BMJ Open* 2014; **4**: e004883 [PMID: 25398675 DOI: 10.1136/bmjopen-2014-004883]
- 56 **Räisänen S**, Lehto SM, Nielsen HS, Gissler M, Kramer MR, Heinonen S. Fear of childbirth predicts postpartum depression: a population-based analysis of 511 422 singleton births in Finland. *BMJ Open* 2013; **3**: e004047 [PMID: 24293208 DOI: 10.1136/bmjopen-2013-004047]
- 57 **Rumbold AR**, Crowther CA. Women's experiences of being screened for gestational diabetes mellitus. *Aust NZ J Obstet Gynaecol* 2002; **42**: 131-137 [PMID: 12069138 DOI: 10.1111/j.0004-8666.2002.00131.x]
- 58 **Silveira ML**, Whitcomb BW, Pekow P, Braun B, Markenson G, Dole N, Manson JE, Solomon CG, Carbone ET, Chasan-Taber L. Perceived psychosocial stress and glucose intolerance among pregnant Hispanic women. *Diabetes Metab* 2014; **40**: 466-475 [PMID: 24948416 DOI: 10.1016/j.diabet.2014.05.002]
- 59 **Singh PK**, Lustman PJ, Clouse RE, Freeland KE, Perez M, Anderson RJ, Vlastos E, Mostello D, Holcomb W. Association of depression with complications of diabetic pregnancy: a retrospective analysis. *J Clin Psychol Med Settings* 2004; **11**: 49-54 [DOI: 10.1023/B: JOCS.000016269.40937.32]
- 60 **Sit D**, Luther J, Dills JL, Eng H, Wisniewski S, Wisner KL. Abnormal screening for gestational diabetes, maternal mood disorder, and preterm birth. *Bipolar Disord* 2014; **16**: 308-317 [PMID: 24164892 DOI: 10.1111/bdi.12129]
- 61 **Song XF**, Liu YJ, Wang WH, Liu YL, Ni CH, Xu ZR. Investigation of depressive symptoms and analysis of related factors in patients with gestational diabetes mellitus. *Zhongguo Linchuang Kangfu* 2004; **8**: 6559-6561
- 62 **Sundaram S**, Harman JS, Cook RL. Maternal morbidities and postpartum depression: an analysis using the 2007 and 2008 Pregnancy Risk Assessment Monitoring System. *Womens Health Issues* 2014; **24**: e381-e388 [PMID: 24981397 DOI: 10.1016/j.whi.2014.05.001]
- 63 **Walmer R**, Huynh J, Wenger J, Ankers E, Mantha AB, Ecker J, Thadhani R, Park E, Bentley-Lewis R. Mental health disorders subsequent to gestational diabetes mellitus differ by race/ethnicity. *Depress Anxiety* 2015; **32**: 774-782 [PMID: 26130074 DOI: 10.1002/da.22388]
- 64 **Whiteman VE**, Salemi JL, Mejia De Grubb MC, Ashley Cain M, Mogos MF, Zoorob RJ, Salihu HM. Additive effects of Pre-pregnancy body mass index and gestational diabetes on health outcomes and costs. *Obesity* (Silver Spring) 2015; **23**: 2299-2308 [PMID: 26390841 DOI: 10.1002/oby.21222]
- 65 **York R**, Brown LP, Persily CA, Jacobsen BS. Affect in diabetic women during pregnancy and postpartum. *Nurs Res* 1996; **45**: 54-56 [PMID: 8570424]
- 66 **Levy-Shiff R**, Lerman M, Har-Even D, Hod M. Maternal adjustment and infant outcome in medically defined high-risk pregnancy. *Dev Psychol* 2002; **38**: 93-103 [PMID: 11806705 DOI: 10.1037/0012-1649.38.1.93]
- 67 **Moore ML**, Meis P, Jeffries S, Ernest JM, Buerkle L, Swain M, Hill C. A comparison of emotional state and support in women at high and low risk for preterm birth, with diabetes in pregnancy, and in non-pregnant professional women. *J Prenat Perinat Psychol Health* 1991; **6**: 109-127
- 68 **World Health Organization**. International Classification of Diseases. Available from: URL: <http://www.who.int/classifications/icd/en/>
- 69 **Mezuk B**, Johnson-Lawrence V, Lee H, Rafferty JA, Abdou CM, Uzogara EE, Jackson JS. Is ignorance bliss? Depression, antidepressants, and the diagnosis of prediabetes and type 2 diabetes. *Health Psychol* 2013; **32**: 254-263 [PMID: 23437855 DOI: 10.1037/a0029014]
- 70 **Golden SH**, Lazo M, Carnethon M, Bertoni AG, Schreiner PJ, Diez Roux AV, Lee HB, Lyketsos C. Examining a bidirectional association between depressive symptoms and diabetes. *JAMA* 2008; **299**: 2751-2759 [PMID: 18560002 DOI: 10.1001/jama.299.23.2751]
- 71 **Pan A**, Lucas M, Sun Q, van Dam RM, Franco OH, Manson JE, Willett WC, Ascherio A, Hu FB. Bidirectional association between depression and type 2 diabetes mellitus in women. *Arch Intern Med* 2010; **170**: 1884-1891 [PMID: 21098346 DOI: 10.1001/

- archinternmed.2010.356]
- 72 **Champaneri S**, Wand GS, Malhotra SS, Casagrande SS, Golden SH. Biological basis of depression in adults with diabetes. *Curr Diab Rep* 2010; **10**: 396-405 [PMID: 20878274 DOI: 10.1007/s11892-010-0148-9]
  - 73 **Lustman PJ**, Anderson RJ, Freedland KE, de Groot M, Carney RM, Clouse RE. Depression and poor glycemic control: a meta-analytic review of the literature. *Diabetes Care* 2000; **23**: 934-942 [PMID: 10895843 DOI: 10.2337/diacare.23.7.934]
  - 74 **Barnard K**, Peveler RC, Holt RI. Antidepressant medication as a risk factor for type 2 diabetes and impaired glucose regulation: systematic review. *Diabetes Care* 2013; **36**: 3337-3345 [PMID: 24065841 DOI: 10.2337/dc13-0560]
  - 75 **Kleinwechter H**, Schäfer-Graf U, Bühner C, Hoesli I, Kainer F, Kautzky-Willer A, Pawlowski B, Schunck K, Somville T, Sorger M. Gestational diabetes mellitus (GDM) diagnosis, therapy and follow-up care: Practice Guideline of the German Diabetes Association(DDG) and the German Association for Gynaecology and Obstetrics (DGGG). *Exp Clin Endocrinol Diabetes* 2014; **122**: 395-405 [PMID: 25014091 DOI: 10.1055/s-0034-1366412]
  - 76 **Kalra B**, Sridhar GR, Madhu K, Balhara YP, Sahay RK, Kalra S. Psychosocial management of diabetes in pregnancy. *Indian J Endocrinol Metab* 2013; **17**: 815-818 [PMID: 24083162 DOI: 10.4103/2230-8210.117216]
  - 77 **Kitzmiller JL**, Block JM, Brown FM, Catalano PM, Conway DL, Coustan DR, Gunderson EP, Herman WH, Hoffman LD, Inturrisi M, Jovanovic LB, Kjos SI, Knopp RH, Montoro MN, Ogata ES, Paramsothy P, Reader DM, Rosenn BM, Thomas AM, Kirkman MS. Managing preexisting diabetes for pregnancy: summary of evidence and consensus recommendations for care. *Diabetes Care* 2008; **31**: 1060-1079 [PMID: 18445730 DOI: 10.2337/dc08-9020]
  - 78 **Huang Y**, Wei X, Wu T, Chen R, Guo A. Collaborative care for patients with depression and diabetes mellitus: a systematic review and meta-analysis. *BMC Psychiatry* 2013; **13**: 260 [PMID: 24125027 DOI: 10.1186/1471-244X-13-260]
  - 79 **Rasmussen B**, Hendrieckx C, Clarke B, Botti M, Dunning T, Jenkins A, Speight J. Psychosocial issues of women with type 1 diabetes transitioning to motherhood: a structured literature review. *BMC Pregnancy Childbirth* 2013; **13**: 218 [PMID: 24267919 DOI: 10.1186/1471-2393-13-218]

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Editorial Board Member of *World Journal of Diabetes*, Amalia Gastaldelli, MSc, PhD, Head of Cardiometabolic Risk Laboratory, Institute of Clinical Physiology, National Research Council, 56100 Pisa, Italy

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## Glucagon-like peptide 1 in the pathophysiology and pharmacotherapy of clinical obesity

Ananthi Anandhakrishnan, Márta Korbonits

Ananthi Anandhakrishnan, Márta Korbonits, Centre for Endocrinology, William Harvey Research Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London EC1M 6 BQ, United Kingdom

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**Correspondence to:** Márta Korbonits, Professor of Endocrinology and Metabolism, Centre for Endocrinology, William Harvey Research Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, Charterhouse Square, London EC1M 6 BQ, United Kingdom. [m.korbonits@qmul.ac.uk](mailto:m.korbonits@qmul.ac.uk)  
Telephone: +44-20-78826238  
Fax: +44-20-78826197

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### Abstract

Though the pathophysiology of clinical obesity is un-

doubtedly multifaceted, several lines of clinical evidence implicate an important functional role for glucagon-like peptide 1 (GLP-1) signalling. Clinical studies assessing GLP-1 responses in normal weight and obese subjects suggest that weight gain may induce functional deficits in GLP-1 signalling that facilitates maintenance of the obesity phenotype. In addition, genetic studies implicate a possible role for altered GLP-1 signalling as a risk factor towards the development of obesity. As reductions in functional GLP-1 signalling seem to play a role in clinical obesity, the pharmacological replenishment seems a promising target for the medical management of obesity in clinical practice. GLP-1 analogue liraglutide at a high dose (3 mg/d) has shown promising results in achieving and maintaining greater weight loss in obese individuals compared to placebo control, and currently licensed anti-obesity medications. Generally well tolerated, provided that longer-term data in clinical practice supports the currently available evidence of superior short- and long-term weight loss efficacy, GLP-1 analogues provide promise towards achieving the successful, sustainable medical management of obesity that remains as yet, an unmet clinical need.

**Key words:** Obesity pathophysiology; Glucagon-like peptide 1 analogues; Glucagon-like peptide 1; Clinical obesity

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**Core tip:** Several lines of clinical evidence implicate an important functional role for glucagon-like peptide 1 (GLP-1) signalling in the pathophysiology of clinical obesity. Here we critically evaluate such findings in way that as yet has been unexplored; using the well established roles of GLP-1 as an incretin and meal to meal satiety signal to go some way toward explaining findings from interventional and observational clinical data that suggest functional deficits of GLP-1 to be a contributor to the obesity phenotype. We also explore

the promise shown by GLP-1 analogues in achieving and maintaining significant weight loss in obese individuals, and use findings to discuss to what extent they too may support a role for GLP-1 in obesity pathophysiology. We conclude by exploring what an association with functional GLP-1 deficit could mean for the clinical management of obesity; conducting cost and risk benefit analyses to evaluate the extent to which GLP-1 analogues may provide a successful and sustainable option for the medical management of obesity that remains as yet, an unmet clinical need.

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## INTRODUCTION

### **Public health and economic impacts of obesity**

Obesity is a global epidemic, perhaps the greatest challenge to global and public health of our time. With a doubling in prevalence from 1980 to 2008<sup>[1]</sup>, 13% of the world's population at present are obese [body mass index (BMI)  $\geq 30$  kg/m<sup>2</sup>] and 39% overweight (BMI  $\geq 27$  kg/m<sup>2</sup>)<sup>[2]</sup>. If recent trends continue, by 2030 up to 57.8% of the world's adult population will be overweight or obese<sup>[3]</sup> (Figure 1). The World Health Organisation (WHO) has estimated that 44% of the global diabetes burden, and 23% and 7%-41% of the burdens for ischaemic heart disease and specific cancers respectively can be attributed to being overweight or obese<sup>[4]</sup>. Psychosocially, stigma and discrimination toward obese people can have consequences for psychological as well as physical health<sup>[5]</sup>, with impaired quality-of life<sup>[6]</sup> and increased rates of depression<sup>[7]</sup> reported in this group. Even modest losses of 5%-10% of total body weight are associated with reduced risk of comorbidities in obese individuals<sup>[8-10]</sup>. Therefore, effectively managing rates of obesity is a major goal in public health policy.

In addition to its physical and psychological burdens, obesity and its comorbidities impose disproportionately high healthcare and economic demands at individual and societal levels<sup>[11]</sup>. Affecting the wider economy indirectly through increased rates of worker illness absenteeism and resultant losses in productivity, healthcare systems are burdened from direct healthcare related costs; obese individuals on average incurring healthcare related costs 30% greater than their healthy weight peers<sup>[12-16]</sup>. A global systematic review has estimated the direct costs of obesity related diseases to account for between 0.7% and 2.8% of a country's total healthcare expenditure<sup>[16]</sup>. In the United Kingdom alone, direct costs to the National Health Service (NHS) of treating overweight and obesity, and related co-morbidities were estimated at £5.1 billion in July 2006; representing around 5% of total

NHS spending<sup>[17,18]</sup>. A computer based micro-simulation model predicting the direct healthcare related costs of overweight and obesity in the United Kingdom should 2001 prevalence remain constant, has forecasted the NHS spending £15.4 billion and £22.5 billion in 2015 and 2050 respectively<sup>[18,19]</sup> on the direct health costs of treating overweight and obesity and related co-morbidities in England alone. An upward trajectory prevented by significant weight loss in those currently obese (Figure 2), findings imply that whilst the prevention of obesity is the strategic imperative, the effective management of those already obese is an immediate priority.

### **Current management of obesity**

Current medical management of obesity involves lifestyle, pharmacological and surgical interventions<sup>[20]</sup>. Lifestyle intervention, in the form of dietary, behavioural and exercise counselling, are currently the suggested first line treatment for obesity; however, whilst a recent meta-analysis reports such interventions to show small but significant benefits on weight loss maintenance, weight loss achieved and sustained with lifestyle intervention alone remains suboptimal<sup>[20-24]</sup>. In the face of such challenges, a number of pharmaceuticals have been marketed to assist weight management over the years<sup>[25,26]</sup> (Table 1). However, adverse effects of some and the transient weight losses associated with others<sup>[27]</sup> mean that the pharmacological management of obesity remains suboptimal. The only proven treatment to achieve and maintain weight loss in obesity is bariatric surgery<sup>[28-30]</sup>. However, surgical and anaesthetic risks associated with overweight and obese status sees these invasive procedures reserved to those patients classed morbidly obese (BMI  $\geq 40$  kg/m<sup>2</sup>) or as a last resort in those failing more conservative management<sup>[20,31,32]</sup>. The minimally invasive and efficacious management of obesity therefore, remains an unmet clinical need.

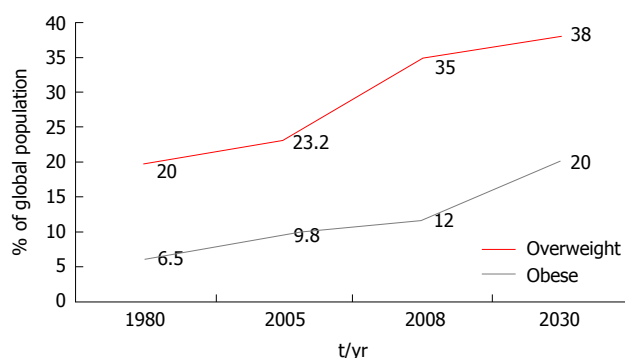
### **Glucagon-like peptide 1 and the management of human obesity**

The ideal management of any illness involves an understanding of its underlying pathophysiology; greater understanding facilitating the development of targeted pharmacotherapies to either replete physiological factors pathologically depleted, or antagonize pathological processes. The pathophysiology of obesity however, remains poorly understood. The WHO has defined the current obesity crisis epidemiologically, as the consequence of an increasing imbalance between energy intake and expenditure<sup>[33]</sup>. Physiologically, energy balance is a closely regulated system involving interactions between peripheral endocrine, nutritional and neural signals acting on regulatory central hypothalamic and hedonic brain regions<sup>[34-36]</sup>. Clinical obesity has been associated with deregulations in both homeostatic and hedonic controls of energy balance potentially facilitated by impaired glucagon-like peptide 1 (GLP-1) signalling<sup>[35-37]</sup> (a role for GLP-1 in the pathophysiology of clinical obesity). Pharmacologically targeting GLP-1

**Table 1** Current and previously Food and Drug Administration licenced anti-obesity pharmacotherapeutics

Drug	Mechanism	Year	Clinical use and limitations	Suspension reason
Currently FDA licenced drugs				
Diethylpropion	NA releasing agent	1959	FDA approved for short term use (3 mo); not recommended with uncontrolled hypertension or heart disease	-
Phentermine	-	1959	FDA and EMA approved for long term use; treatment dependent weight loss	-
Orlistat (Xenical)	-	1999	-	-
Orlistat (Alli)	Pancreatic lipase inhibitor	2007	-	-
Phentermine-topiramate (Qysmia)	-	-	Approved for long term use; treatment dependent weight loss	-
Lorcaserin (Belviq)	5HT <sub>2c</sub> -R antagonist	2012	FDA approved for long term use, recommended in those with cardiovascular disease; treatment dependent weight loss	-
Liraglutide (Saxenda)	GLP-1 analogue	2014	FDA and EMA (2015) approved	-
Previously FDA licenced drugs				
Dinitrophenol	Unknown	1938	-	Dermatitis, neuropathy, agranulocytosis, visual impairment, death
Aminorex	Unknown	1968	-	Chronic pulmonary hypertension
Amphetamines	Monoamine reuptake inhibitor	1971	-	Addiction, hypertension, myocardial toxicity
Fenfluramine	Serotonin reuptake inhibitor	1997	-	Valvular heart disease
Phenylpropanolamine	NA-R and DA-R agonist	2000	-	Haemorrhagic stroke
Rimonabant	CB <sub>1</sub> R antagonist	2009	-	Psychiatric disorders, depression, suicidal ideation
Sibutramine	Serotonin-NA reuptake inhibitor	2010	-	Risk of major cardiovascular events

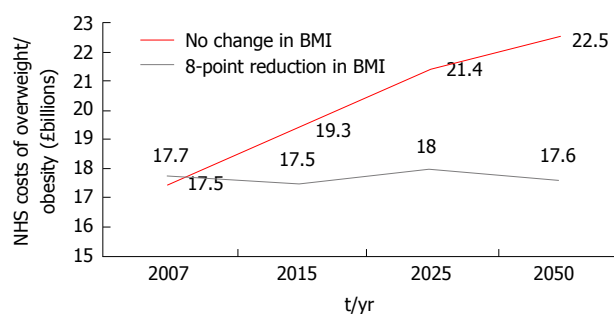
The pancreatic lipase inhibitor Orlistat and GLP-1 analogue liraglutide are the only currently UK licenced anti-obesity agents). 5HT<sub>2c</sub>: Serotonin receptor; NA: Noradrenaline; DA: Dopamine; CB<sub>1</sub>R: Cannabinoid receptor; R: Receptor; FDA: Food and Drug Administration; EMA: European Medical Association.



**Figure 1** Global overweight and obesity trends and projections. If recent trends continue unabated, by 2030, 38% and 20% of the world's adult population are projected to be overweight or obese respectively<sup>[8]</sup>.

therefore, may go some way towards achieving the successful and sustainable medical management of clinical obesity that as yet remains to be achieved.

GLP-1 is a 31 amino acid polypeptide primarily synthesized by the enteroendocrine L cells of the terminal ileum. Amongst its pleotropic central and peripheral effects, GLP-1 acts as a potent incretin first clinically used in the medical management of overweight or obese individuals with type 2 diabetes mellitus (T2DM)<sup>[38]</sup>. The repeatedly demonstrated ability of GLP-1 analogues to induce weight loss in this cohort<sup>[39,40]</sup> prompted phase



**Figure 2** Projected trends in National Health Service costs from a micro-simulation model. A computer based micro-simulation model<sup>[18]</sup> predicted the direct healthcare related costs of overweight and obesity in the United Kingdom at five time points from 2001 (2001, 2007, 2015, 2025 and 2050) should prevalence of overweight and obesity remain constant (red) or if an average of an 8-point BMI reduction was achieved in all those obese in 2001 (grey). BMI: Body mass index; NHS: National Health Service.

III trials studying the weight loss efficacy of the GLP-1 analogue liraglutide (3 mg; trade name Saxenda) vs placebo<sup>[41]</sup> and the pancreatic lipase inhibitor orlistat<sup>[42,43]</sup> (the only anti-obesity drug licensed in the United Kingdom) in non-diabetic overweight and obese adults. The greater weight loss efficacy achieved and maintained by GLP-1 analogues prompting the Food and Drug Administration (FDA) in 2014 to approve Saxenda as the first GLP-1 analogue for use as a weight loss aid in obese

adults and overweight adults with at least one weight related co-morbidity<sup>[44]</sup>. March 2015 saw the European Medical Association (EMA) grant marketing authorization for 3 mg liraglutide under the FDA approved criteria in all 28 European Union (EU) states<sup>[45]</sup>. However, launching in April 2015 in the United States at a cost of over \$1000 per patient a month, cost-benefit is of greater issue in EU nations such as the United Kingdom where health care is primarily socially funded; undoubtedly contributing to the uncertainty of launch plans in the United Kingdom at present<sup>[46]</sup>. Clinical evidence however implicates a role for functional impairments in GLP-1 signalling in the pathophysiology of obesity, GLP-1 agonism therefore may be the first truly targeted therapeutic in the medical management of clinical obesity. Therefore, with its superior clinical efficacy to currently United Kingdom licensed therapies benefiting patients through greater achieved and maintained weight loss and the economy through the potential to reduce long-term financial burdens of obesity, the cost-benefit spectrum may therefore be swayed, favouring the use of GLP-1 analogues in the medical management of obesity in the United Kingdom<sup>[46]</sup>.

## THE HOMEOSTATIC AND HEDONIC CONTROL OF ENERGY BALANCE

Physiologically, energy balance is a closely regulated system involving interactions between peripheral endocrine, nutritional and neural signals acting on regulatory central hypothalamic<sup>[34]</sup> and hedonic<sup>[35,36]</sup> brain regions. Where previously the neurocircuits mediating the homeostatic and hedonistic control of energy balance were considered distinct entities, it has now emerged that considerable cross talk exists with implications for the pathophysiology of clinical obesity.

### Peripheral afferents

Peripheral signals involved in energy homeostasis are often stratified as long or short acting. Long acting signals provide information about available energy stores, and in response, the brain makes corrective adjustments to food intake and energy expenditure to maintain body weight<sup>[47]</sup>. The white adipocyte hormone leptin<sup>[48]</sup> and pancreatic hormone insulin are the two major afferents governing long-term energy balance and act primarily as anorexigens. Food intake and energy expenditure in the short term are modulated by a wide variety of situational and meal-related factors, among the most important are short-term gut derived hormones such as GLP-1 that act to signal acute energy status. Originally thought to exert their effects on energy balance through modulating homeostatic hypothalamic circuits, both long and short term afferents may also modulate the hedonic drive toward food consumption, though these pathways remain less extensively studied<sup>[49]</sup> (Figure 3).

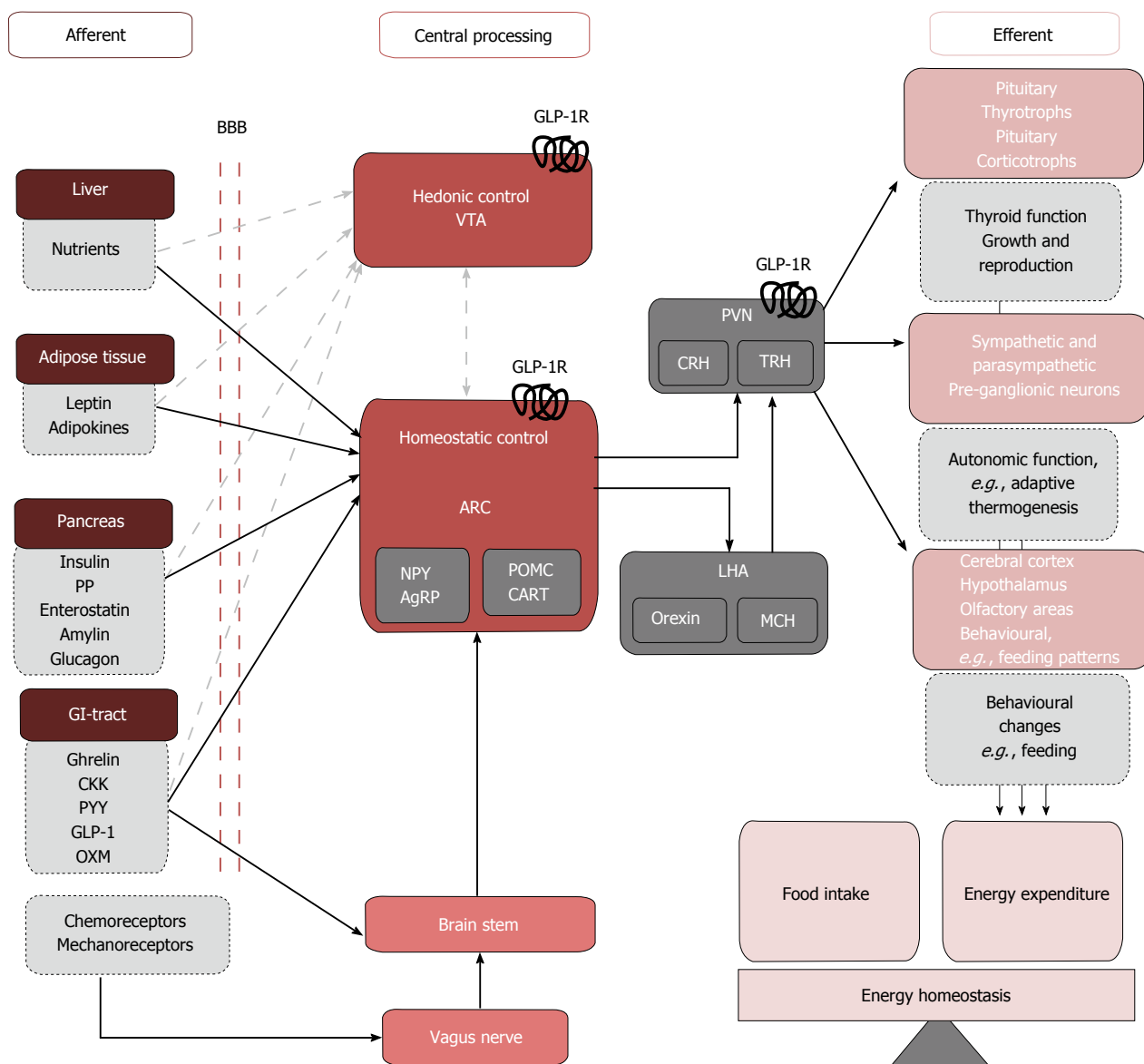
### Central controllers

The homeostatic control of food intake: The hypothalamic

arcuate nucleus (ARC) is believed to play a crucial role in the homeostatic control of energy balance. At a cellular level, the ARC contains two distinct neural populations exerting antagonistic effects on food intake; a medially located orexigenic (appetite stimulating) population consisting of neurons co-expressing Agouti related peptide (AgRP) and neuropeptide Y and a laterally located anorexigenic (appetite suppressing) population consisting of neurons co-expressing pro-opiomelanocortin (POMC) and cocaine and amphetamine related transcript (CART)<sup>[55-58]</sup>. Both neural subsets project to melanocortin 4 receptor (MC4R) positive neurons located in intra- and extra-hypothalamic sites. POMC is cleaved to produce  $\alpha$ -MSH an agonist of MC4R whereas AgRP acts an inverse agonist<sup>[59-61]</sup>. The ARC may exert its effects on energy homeostasis by direct cortical projections or indirectly *via* second order neurons in adjacent hypothalamic nuclei of which the paraventricular nucleus (PVN) is believed to be play a crucial role<sup>[62,63]</sup>. GLP-1 receptors (GLP1-Rs) have been localized pre-clinically in the ARC and PVN<sup>[50,51]</sup> and stimulation of these receptors reduce food intake to induce weight loss in rodents. Targeting the homeostatic controls of energy balance may therefore be the means by which GLP-1 agonism achieves its weight loss effects in the clinic, suggesting an underlying deregulation in GLP-1 signalling contributing to the multifactorial pathophysiology of human obesity.

**The hedonic control of food intake:** Despite a robust homeostatic system governing energy balance, feeding and meal termination are also influenced by hedonic, reward-related factors such as palatability and the perceived rewards associated with meal consumption. The drive to pursue such pleasurable experiences largely mediated by the mesolimbic rewards system originating from dopaminergic neurons in ventral tegmental area (VTA) that terminate on neurons in the nucleus accumbens. Though the relationship between peripheral afferents signalling acute and long term energy status and central hedonic control centres are less well defined, GLP-1Rs have been located in the dopaminergic neurons of the VTA<sup>[64]</sup> where activation inhibits neural firing, potentially reducing hedonic drives toward food consumption. Interestingly, where the homeostatic control of energy balance modulates food intake to regulate the amount of body fat an individual maintains<sup>[65]</sup>, in obesity, despite an overall positive energy balance, hyperphagia is the norm. Where previously, the neurocircuits mediating the homeostatic and hedonistic control of energy balance were considered distinct entities, it has now emerged that considerable cross talk exists and central GLP-1 signalling has been implicated as a mediator of such interactions (detailed in a number of excellent reviews<sup>[36,37,54]</sup>). A skew toward hedonic and away from homeostatic controls of energy balance may explain the pathological hyperphagia seen in obesity; restoring the balance between homeostatic and hedonic drives towards food consumption may therefore be the means by which GLP-1 agonism achieves its sustained





**Figure 3 The hedonic and homeostatic controls of energy balance.** Peripheral signals from the Liver, adipose tissue pancreas, GI-tract cross the BBB to directly signal to neurons of the ARC of the hypothalamus. GI-tract enteroendocrine hormones and chemo- and mechanoreceptor neural afferents can also indirectly activate the ARC via the vagus nerve and brainstem. The net output of the ARC neurons is relayed to second order intrahypothalamic neurons in the PVN, and LHA that express the MC4R. GLP-1Rs have been localized pre-clinically in the ARC and PVN<sup>[50,51]</sup>, stimulation of these receptors inducing reductions in food intake and weight loss potentially through efferent pathways that involve the activation of TRH and CRH expressing neurons and pre-ganglionic sympathetic and parasympathetic neurons. Feeding and meal termination are also influenced by hedonic, reward-related factors centrally processed in the VTA. Though the interactions between peripheral nutrient signals and rewards neurocircuitry are not extensively defined (grey dashed arrows) GLP-1Rs have been localized pre-clinically in the VTA<sup>[52]</sup>. Previously considered as separate entities, severe cross-interactions exist between central homeostatic and hedonic control centres<sup>[53]</sup>. This communication may be mediated by central GLP-1 signalling<sup>[36,37,54]</sup>. GI-tract: Gastrointestinal tract; BBB: Blood-brain barrier; ARC: Arcuate nucleus; PVN: Paraventricular nucleus; LHA: Lateral hypothalamic area; MC4R: Melanocortin 4 receptor; TRH: Thyrotrophin releasing hormone; CRH: Corticotrophin releasing hormone; VTA: Ventral tegmental area; PP: Pancreatic polypeptide; CCK: Cholecystokinin; PYY: Polypeptide-YY; OXM: Oxymodulin.

weight loss effects in the clinic, suggesting an underlying deregulation in GLP-1 signalling contributing to the multifactorial pathophysiology of human obesity.

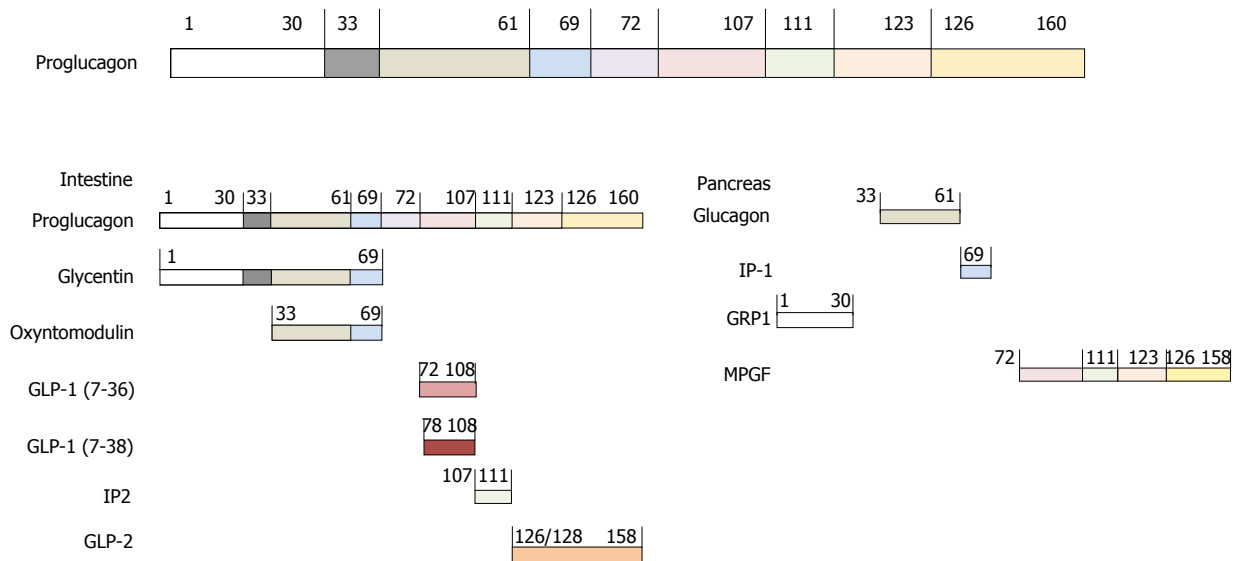
## GLP-1

### Synthesis, secretion and degradation

GLP-1 is a 31 amino acid polypeptide derived from post-translational processing of the native 160 amino acid peptide proglucagon by the enzyme prohormone convertase 1 (PC1/3). Peripheral proglucagon gene

expression has been localized to the enteroendocrine L cells and pancreatic  $\alpha$ -cells whilst centrally, proglucagon expressing neurons have been localized to brainstem regions such as the nucleus of the solitary tract (NTS)<sup>[66-68]</sup>. Tissue specific post-translational processing liberates different pro-glucagon derived peptides<sup>[69]</sup> depending on subtype of PC enzyme present. Figure 4 details the different post-translational products following PC1/3 and 2 cleavage.

GLP-1 is primarily synthesized by PC1/3 activity in the intestinal L cells<sup>[75]</sup>; open-type epithelial cells



**Figure 4 Post-translational tissue specific processing of proglucagon<sup>[70-72]</sup>** The 160 amino acid pro-glucagon gene (GCG) encoded on chromosome 2 undergoes tissue specific post-translational cleavage by prohormone convertase (PC) 1/3 and PC2 in central and peripheral sites. This figure shows the major cleavage products of the GCG with numbers indicating the amino acids between which the hormone product lies and at which the PC enzymes act. In the pancreas, PC2 dominates and liberates glucagon. In the intestine PC 1/3 activity dominates and produces GLP-1, of note, the other products of proglucagon cleavage by PC1/3 are produced in a 1:1 ratio with GLP-1<sup>[73,74]</sup>. The PC responsible for cleaving GCG in the central nervous system is not well established; both PC1/3 and PC2 may play a role. GLP: Glucagon-like peptide; GRP1: Gastrin-releasing peptide 1; IP2: Intervening peptide 1/2.

most densely located in the ileum and colon<sup>[76-78]</sup>. Long apical processes that extend toward the intestinal lumen<sup>[77]</sup> allow direct nutrient sensing by L cells, of which glucose has been implicated as the most potent GLP-1 secretagogue in both healthy and T2DM humans<sup>[79]</sup>. Being in close proximity to neurons of the enteric nervous system and the intestinal microvasculature<sup>[80,81]</sup>, L cells also receive neural and hormonal signals that act as indirect nutrient sensors. Following synthesis, GLP-1 is secreted from the L-cells *via* secretory granules located in the basolateral membrane. GLP-1 secretion in response to nutrient sensing is biphasic; an initial rapid rise occurring within 10-15 min post-prandial, followed by a second longer phase peaking at 30-60 min<sup>[82]</sup>. The early phase of GLP-secretion has traditionally been attributed to signals from the parasympathetic vagal nerve and neurotransmitters such as gastrin-releasing peptide (GRP) and acetylcholine. However, more recently, GLP-1 secreting cells that show direct secretory responses to nutrient stimulation have been localised in significant numbers in the proximal small intestine implicating a role for this albeit sparser population of proximal GLP-1 releasing cells in the rapid postprandial rises of plasma GLP-1<sup>[83-85]</sup>. The second phase is mediated *via* direct nutrient contact with subsequent membrane depolarization or activation of second messenger systems mediating GLP-1 release. Figure 5 depicts the major nutrient, neural and hormonal secretagogues of GLP-1.

Secreted GLP-1 is rapidly degraded at its N-terminal residue by the ubiquitously expressed enzyme dipeptidyl peptidase IV (DPPV) to yield residues GLP-1 (9-36 amide) and GLP-1 9-37<sup>[88,89]</sup>. The majority of GLP-1 degradation is attributed to membrane-bound DPPV in the hepatic portal system resulting in an extremely short half-life (about 2 min)<sup>[81,90]</sup>. As such, only about 10%-15%

of GLP1 secreted from intestinal L cells reaches peripheral downstream targets. The amount of GLP-1 reaching potential central targets involved in energy balance is unknown. As parenteral administration of GLP-1 avoids the physiological first-pass effect of hepatic DPPV, the supraphysiological plasma concentrations achieved by subcutaneous (SC) administration may explain the weight loss efficacy achieved by 3 mg liraglutide in obese and overweight patients in the clinic. Findings also go some way to suggest either a reduction in secretion of, or sensitivity to, physiological GLP-1 secretion as a contributor to the multifactorial pathophysiology of human obesity.

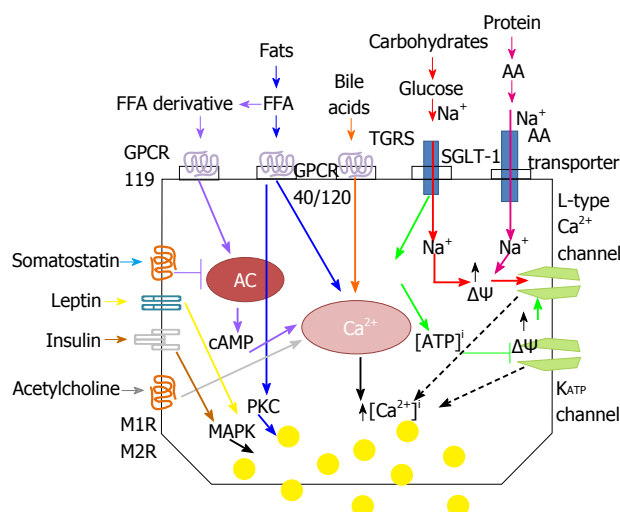
### Central and peripheral effects of GLP-1

GLP-1 exerts its effects by intracellular signalling pathways activated after binding to the G-protein coupled receptor GLP-1R<sup>[91]</sup>. The extensive central and peripheral expression of the GLP-R reflects the pleiotropic physiological roles of GLP-1 that are summarised in Figure 6 and extensively reviewed elsewhere<sup>[70,86]</sup>. From this point onward the review will focus on exploring the evidence surrounding a role for physiological GLP-1 signalling in the regulation of energy balance and deregulations of this signalling as one contributor to the multifactorial pathophysiology of clinical obesity.

## GLP-1 AND THE REGULATION OF ENERGY BALANCE

### Evidence: Effects of GLP-1 administration on food intake and energy expenditure in man

Numerous clinical studies have examined the relationship between acute physiological and supraphysiological



**Figure 5 Mechanisms of glucagon-like peptide 1 release from enteroendocrine L cell.** Glucagon-like peptide 1 (GLP-1) release from L-cells is regulated by direct nutrient sensing *via* receptors and channels on apical processes or indirectly *via* neuro-hormonal mechanisms<sup>[70,71,86,87]</sup>. **A:** Nutrient signals. Carbohydrates: Glucose derived from carbohydrate metabolism is the most potent stimuli for GLP-1 secretion. Glucose can trigger GLP-1 release by two mechanisms: (1) the sodium-glucose cotransporter-1 (SGLT-1) couples the transport of glucose with Na ions. Na<sup>+</sup> influx leads to membrane depolarization ( $\Delta\Psi$ ) (red arrows); and (2) glucose metabolism generates adenosine triphosphate (ATP). Elevated intracellular ATP concentrations [ATP]<sup>i</sup> close KATP channels and leads to membrane depolarization ( $\Delta\Psi$ ) (green arrows). Both routes to membrane depolarisation increase intracellular Ca levels ([Ca<sup>2+</sup>]<sup>i</sup>) by opening L-type Ca channels. Elevated [Ca<sup>2+</sup>]<sup>i</sup> triggers the exocytosis of GLP-1 secretory granules located at the basolateral surface of the enteroendocrine L cell (dashed lines). Fats: Fats are potent stimuli for GLP-1 secretion. Free fatty acids (FFA) (blue arrows) interact with G-protein coupled receptors (GPCRs) that trigger Ca<sup>2+</sup> release from internal stores and also activate protein kinase C (PKC). FFA derivatives (purple arrows) interact with GPCRs that activate second messenger systems involving adenylate cyclase (AC) and cyclic AMP (cAMP) which increases [Ca<sup>2+</sup>]<sup>i</sup>. Bile acids (orange arrows) and short chained fatty acids (not shown) also increase [Ca<sup>2+</sup>]<sup>i</sup> by GPCR interactions. Proteins: Protein is a weak stimulator of GLP-1 release when compared with sugars and lipids. Amino acids (AA) derived from protein breakdown are transported intracellularly with Na<sup>+</sup> *via* Na<sup>+</sup> dependent AA transporters. Na<sup>+</sup> influx causes membrane depolarization and elevated [Ca<sup>2+</sup>]<sup>i</sup> with resultant GLP-1 exocytosis (pink arrows); **B:** Hormonal signals. Somatostatin inhibits GLP-1 release by blocking AC activation (light blue arrows). The peripheral adiposity signals leptin (yellow arrows) and insulin (brown arrows) are thought to stimulate GLP-1 release *via* activation of mitogen-activated protein kinase (MAPK) signalling pathway; **C:** Neural signals. Acetylcholine binding to muscarinic receptors (M1R, M2R) elevates [Ca<sup>2+</sup>]<sup>i</sup> stimulating GLP-1 release (grey arrows). GRP is thought to stimulate GLP-1 release in association with the activation of mitogen activated protein kinase kinase (MAPKK) and subsequent phosphorylation and activation of MAPK (not shown).

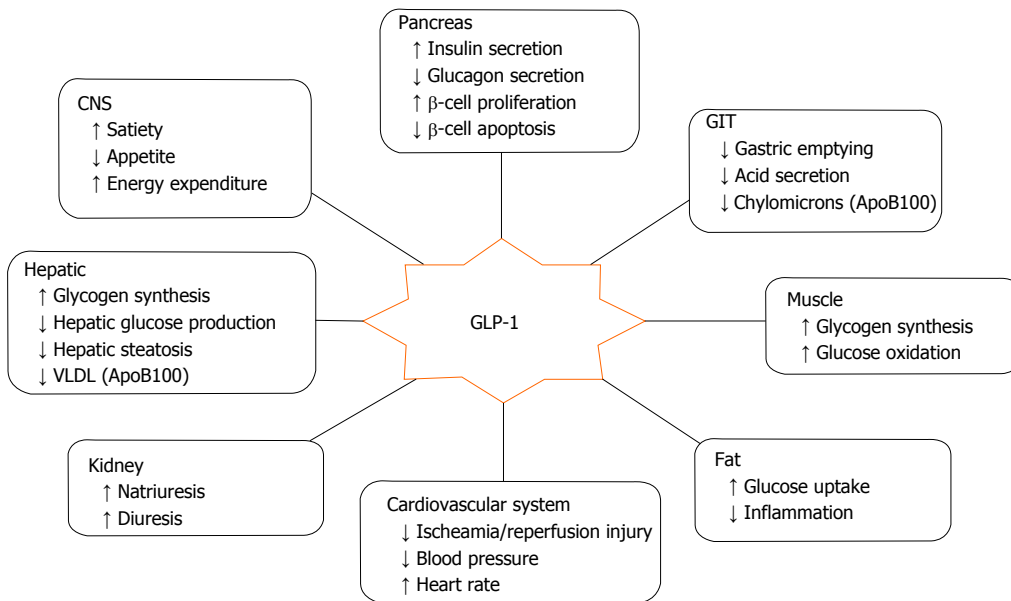
doses of GLP-1 with measurements of food intake and feelings of hunger and satiety in healthy normal weight and obese adults with and without T2DM<sup>[92-99]</sup>. The main findings of these studies have been summarized in Figure 7. Though individual studies are conflicting, a meta-analysis reports that acute GLP-1 infusion induces a mean 11.7% decrease in food intake when compared with saline control in man<sup>[100]</sup>. Interestingly, whilst supra-physiological doses of GLP-1 reduces appetite and food intake in both lean and obese subjects, physiological GLP-1 doses reduces appetite and food intake in only lean subjects<sup>[93,94,97,99]</sup>. Findings go some way to suggest

a role for resistance to physiological GLP-1 signalling as a factor contributing to obesity pathophysiology. Interestingly, whilst physiological GLP-1 infusions in obese subjects induce appetite reductions<sup>[92,95]</sup> similar to those observed in their lean peers, this is not translated into a reduction in food intake, suggesting pathological alterations of GLP-1 signalling in obesity that reinforce feeding despite a reduced physiological drive to food intake. One mechanism that this may be achieved is through a pathological skew toward hedonic and away from homeostatic controls of energy balance in obesity, potentially mediated by deregulated central GLP-1 signalling (a role for GLP-1 in the pathophysiology of clinical obesity).

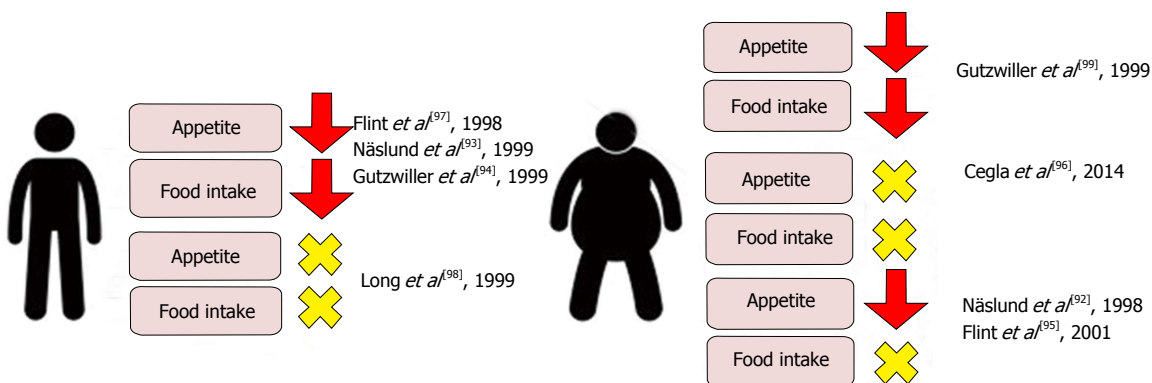
Whilst evidence from clinical interventional studies suggests that physiological GLP-1 contributes to negative energy balance by decreasing food intake. The effects of GLP-1 on energy expenditure are less clear. Fasting plasma GLP-1 concentrations have been positively associated with increased rates of energy expenditure in man<sup>[101]</sup>. Clinical evidence regarding the effects of acute GLP-1 administration on energy expenditure however is conflicting. Physiological infusions of GLP-1 have been reported to reduce energy expenditure in lean and non-diabetic obese patients<sup>[95,102]</sup> associated with reduced carbohydrate metabolism. Others, however, have observed that supraphysiological infusions of GLP-1 increase energy expenditure in lean individuals in an insulin dependent manner<sup>[103]</sup>.

### Interpretations: Implications for the clinic

Evidence from clinical interventional studies suggests that acute post-meal rises in GLP-1 contribute to negative energy balance primarily through an anorexigenic effect. The long-acting GLP-1 analogue liraglutide (3 mg) has recently been approved as a once daily bolus SC injection for the medical management of obesity. The sustained anorectic effect of a long term agonist combined with supra-physiological dosing perhaps the mechanism of the clinical weight loss efficacy achieved by liraglutide 3 mg. Unfortunately, to date, clinical studies assessing the comparative efficacy of acute vs continuous GLP-1 administration on appetite reduction and weight loss remain scarce. Näslund *et al.*<sup>[104]</sup> compared the effects of 4 doses of acute GLP-1 infused 30 min prior to meals [prandial subcutaneous infusion (PSI)] to an equivalent dose of continuous SC GLP-1 infusion (CSI) on food intake and weight loss in non diabetic obese patients. Though both acute and continuous GLP-1 infusion produced significant reductions in food intake when compared to placebo ( $P = 0.02$  PSI and CSI), a statistically significant weight loss compared to placebo was only observed following PSI. With respect to the clinic, findings suggest that lowered dose; more frequent GLP-1 administration may prove more efficacious in inducing weight loss in obese patients. Nevertheless, in view of the negative impact of SC drug administration on patient adherence and the potential biases associated with the



**Figure 6 Central and peripheral effects of glucagon-like peptide 1.** *Ex vivo* and *in vivo* studies in rodents, and observational and interventional studies in man have allowed the characterization of numerous central and peripheral effects of GLP-1. Peripheral effects of GLP-1 may be classed broadly as pancreatic or extra-pancreatic. Pancreatic effects of GLP-1 act to promote insulin secretion (incretin effect). Extra-pancreatic effects of GLP-1 include: (1) regulation of energy metabolism and nutrient storage (liver, muscle and fat); (2) efficient nutrient handling (stomach and GIT); and (3) others: Cardiovascular repair, blood pressure control, diuresis<sup>[86]</sup>. VLDL: Very low-density lipoproteins; GLP-1: Glucagon-like peptide 1; GIT: Gastrointestinal tract; CNS: Central nervous system.



**Figure 7 Effects on Visual Analogue Scale assessed appetite scores and ad libitum food intake in lean and obese subjects following physiological and supraphysiological infusions of glucagon-like peptide 1.** Though individual studies report conflicting data, a meta-analysis of clinical studies evaluating the acute effects of GLP-1 infusion on food intake reports a mean 11.7% decrease when compared with saline control<sup>[100]</sup>. GLP-1: Glucagon-like peptide 1.

significantly greater peak plasma GLP-1 concentrations achieved following PSI compared to CSI (269.4 pmol vs 88.7 pmol) once daily bolus administration at present, seems to be the most clinically efficacious means of therapeutic GLP-1 analogue delivery.

#### **Interpretations: Potential effectors of GLP-1s negative energy balance effects**

Clinical and pre-clinical evidence suggests that targeting peripherally and centrally located GLP-1Rs may exert the anorectic effects of physiological GLP-1 signalling.

**Peripheral effectors:** Histological studies in man have shown GLP-1Rs to be expressed in cells of the gastric mucosa and in pancreatic islet cells<sup>[105,106]</sup>. Pre-clinically, stimulation of gastric and pancreatic GLP-1Rs are associated with reductions in food intake that

occurs alongside activation of hedonic and homeostatic brain regions<sup>[47,63,86]</sup>. Findings suggest physiological GLP-1 signalling may induce its anorectic effects in man by indirectly activating central controllers of appetite through gastric and pancreatic receptors.

Gastric mechanoreceptors are activated by gastric distension following acute nutrient intake, and gastric mechanoreceptor signalling plays an important role as a meal-to-meal satiety signal, activating the NTS which in turn modulates neural activity in both the ARC the VTA<sup>[107]</sup> (the homeostatic and hedonic control of energy balance). By relaying to the NTS, mechanoreceptor induced anorectic effects may therefore be exerted through modulation of both homeostatic and hedonic appetite control. The amount of gastric distension in response to a given meal is negatively associated with the rate of gastric emptying; delayed gastric emptying



positively associated with increased satiety and fullness in both healthy and obese patients<sup>[108-112]</sup>. GLP-1 has been found to delay gastric emptying in healthy lean, obese and T2DM subjects, and histological studies in man have shown that GLP-1Rs are expressed in gastric mucosa<sup>[92,105,113-116]</sup>. Post-prandial GLP-1 secretion may therefore exert its anorectic effect through activating GLP-1Rs in gastric mucosa, which in turn increase mechanoreceptor firing and signalling to the NTS. Though the neurotransmitters involved in relaying signals from the NTS to homeostatic and hedonic appetite controls remain to be defined, physiological gastric distension in rodents has been shown to up-regulate *GLP-1* gene expression in the NTS associated with central proglucagon processing<sup>[117]</sup>, implicating a role for centrally synthesised GLP-1.

In the fasted state, the stomach is empty and so gastric motility is reduced to basal levels. That reductions in appetite after GLP-1 administration have been observed in fasting human subjects<sup>[99]</sup>, suggests that mechanisms other than delaying gastric motility contribute to the physiological anorectic effect of GLP-1. The glucoregulatory hormone insulin, traditionally viewed as an anorectic signal involved in the regulation of long-term energy balance<sup>[47,63]</sup>, displays both basal and acute meal-related secretion<sup>[118]</sup>. With acute insulin administration associated with reduced *ad libitum* food intake in healthy lean individuals<sup>[119]</sup>, findings implicate a role for insulin as an anorexigen involved in the regulation of short term energy balance. Insulin receptors are widely expressed in the ARC and VTA<sup>[120-122]</sup>, thus modulation of both homeostatic and hedonic appetite control may be the means by which insulin exerts its anorectic effects on short-term energy balance.

The most extensively studied of GLP-1's physiological roles is as a positive modulator of insulin secretion from pancreatic  $\beta$ -cells<sup>[123]</sup> (evidence: Effects of GLP-1 administration on food intake and energy expenditure in man). Whilst GLP-1 has been shown to increase energy expenditure in healthy lean individuals in an insulin dependent manner<sup>[103]</sup>, no clinical evidence to date exists exploring the role of insulin as a mediator of GLP-1 anorexigenic signalling. Studies assessing the effects of GLP-1 interactions with the orexigen ghrelin however, suggest that this may indeed be the case. Ghrelin receptors have been localised preclinically in *Agrp/NpY* neurons of the ARC and dopaminergic neurons of the VTA<sup>[124,125]</sup>, with activation of neurons in either brain region producing orexigenic effects. GLP-1 infusion in healthy lean humans is associated with significant suppression of postprandial rises in ghrelin<sup>[126]</sup>; the decline in orexigenic signalling a potential indirect mediator of GLP-1's anorexigenic effect. Interestingly, the reductions in ghrelin concentration observed with GLP-1 infusion inversely correlate with coinciding rises in insulin concentration and elsewhere, insulin infusion has been shown to display a reciprocal relationship with ghrelin secretion in man<sup>[127]</sup>. Together, findings suggest that

GLP-1's anorectic effects may be mediated secondary to its incretin effect that in turn suppresses ghrelin release, thus orexigenic signalling.

**Central controllers:** Histological and *in vivo* studies in rodents have shown that GLP-1Rs are expressed in anorexigenic POMC/CART neurons of the ARC and in dopaminergic neurons of the VTA<sup>[59,61,18,128]</sup> where they stimulate and inhibit neural firing respectively. Preclinical studies have shown that the stimulation of the POMC/ARC neurons of the hypothalamus and inhibition of the dopaminergic neurons of the VTA reduce food intake. Findings suggest that GLP-1 may exert its negative energy balance effects in man through direct activation of central GLP-1 receptors in the ARC and VTA; activating the anorexigenic homeostatic and inhibiting the hedonic hyperphagic drives to food intake. With the development of neuroimaging techniques, *in vivo* clinical studies substantiate the effects of GLP-1 on brain regions involved in the homeostatic and hedonic controls of energy balance. Whether these effects are mediated by direct central GLP-1R activation or indirectly *via* peripherally located GLP-1Rs however, remain to be defined.

Using fluorodeoxyglucose positron emission tomography Alvarez *et al.*<sup>[129]</sup> demonstrate that GLP-1 infusion in lean individuals reduces glucose metabolism in the hypothalamus and brainstem. With patients fasted during the study and with no changes in peripheral hormone profiles observed, the effects of gastric mechanoreceptor activation or other hormonal influences respectively on observed effects are negated. Elsewhere, correlations between PET assessed increases in hypothalamic blood flow and physiological post-prandial rises in serum GLP-1 have been observed<sup>[130]</sup>. Both findings may represent altered neural activity in brain regions associated with homeostatic energy balance secondary to direct or indirect GLP-1/GLP-1R signalling. The effects of this alteration in central neural activity on food intake and appetite however, have not been explored. Using functional magnetic resonance imaging (fMRI), De Silva *et al.*<sup>[131]</sup> demonstrate that GLP-1 infusion in lean individuals attenuates neuronal activity in 6 brain regions involved in rewards processing and hedonic feeding accompanied with reductions in food intake. Though neither parameter reached statistical significance vs placebo, results support the idea that central GLP-1 signalling may at least in part exert its negative energy balance effects through modulations in hedonic appetite control centres, potentially by reducing the hedonic value associated with food and food-driven motivation.

Clinical evidence exists to suggest that the SNS modulates energy expenditure through increased thermogenesis assessed *in vivo* as muscle sympathetic nerve activity (MSNA)<sup>[132,133]</sup>; increased MSNA positively associated with increased short and longer term energy expenditure in otherwise healthy human subjects<sup>[134,135]</sup>. Peripheral GLP-1 infusion has been shown to significantly

**Table 2 Obesity and endocrine phenotypes in probands with *PCSK1* gene deletion monogenic obesity**

Ref.	Jackson <i>et al</i> <sup>[144]</sup> , 1997	Jackson <i>et al</i> <sup>[143]</sup> , 2003	Farooqi <i>et al</i> <sup>[148]</sup> , 2007	Frank <i>et al</i> <sup>[145]</sup> , 2013	Parker <i>et al</i> <sup>[147]</sup> , 2013	Bandsma <i>et al</i> <sup>[146]</sup> , 2013
Obesity phenotype						
Hyperphagic, early-onset	Yes	Yes	Yes	Yes	Yes	Yes
Endocrine phenotype						
Abnormal glucose metabolism	Yes	Yes		Yes	Yes	Yes
Hypogonadotrophic hypogonadism	Yes			Yes	Yes	
Hypocortisolaemia	Yes	Yes		Yes	Yes	
Hypothyroidism			Yes	Yes	Yes	
Central diabetes insipidus					Yes	
Others						
Early onset malabsorptive diarrhoea	Yes	Yes	Yes	Yes	Yes	Yes

Proband details: Jackson *et al*<sup>[144]</sup> (1997) - a 40-year-old Caucasian woman; Jackson *et al*<sup>[143]</sup> (2003) - female Caucasian infant, non consanguineous; Farooqi *et al*<sup>[148]</sup> (2007) - 6 North African boy, consanguineous; Frank *et al*<sup>[145]</sup> (2013) - male infant; Parker *et al*<sup>[147]</sup> (2013) - 13 children aged 3 to 17; Bandsma *et al*<sup>[146]</sup> (2013) - 2 children age 2 and 7 Arab, consanguineous, 2 children aged 1 and 10, African, non-consanguineous.

increase MSNA in healthy human controls<sup>[136]</sup> and suggest that GLP-1 signalling may produce its negative energy balance effects not only through anorexigenic signalling, but also by increasing energy output.

## A ROLE FOR GLP-1 IN THE PATHOPHYSIOLOGY OF CLINICAL OBESITY

### Genetics

Genetic analyses in man suggest clinical obesity is associated with a lack of functional GLP-1 signalling that may contribute to the development of the obesity phenotype.

**Monogenic human obesity:** Monogenic human obesity is a rare form of clinical obesity that shows Mendelian patterns of inheritance; the obesity phenotype attributed to the loss or gain of function in a single gene<sup>[137]</sup>. Two broad classes of Mendelian human obesity exist; syndromic obesity encompasses about 30 Mendelian disorders wherein obesity co-presents alongside characteristic physical and developmental anomalies. Though causative genes have been identified, the mechanisms through which the genetic mutations induce obesity are not completely understood in all cases<sup>[138]</sup>. Non-syndromic obesity is characterized by a severe, early onset hyperphagic obesity attributed to loss of function mutations in 1 of 11 genes<sup>[139-141]</sup>. Interestingly, 8 of these genes have physiological roles in the central control of energy balance<sup>[142]</sup>. One such gene is PCSK1 encoding the enzyme PC1/3 involved in the proteolytic processing of proglucagon, to yield, amongst other peptides, GLP-1 (GLP-1). Six studies to date document the relationship between autosomal recessive, compound heterozygous or homozygous<sup>[143-148]</sup> mutations in PCSK1 in 21 probands associated with reduced or absent function of PC1/3. Table 2 details the phenotypes of probands, all of whom presented with an early onset hyperphagic obesity and malabsorptive diarrhoea with varying, though extensively

overlapping endocrine phenotypes.

Though the cause of the obesity and endocrine phenotypes associated with PCSK1 mutation are unknown, they may well be attributed to the loss of PC1/3 pro-hormone processing function. Signs of impaired intestinal<sup>[146]</sup> pro-glucagon processing have been described in probands with PC1/3 deficiency and may contribute to the development of the obesity phenotype secondary to reduced GLP-1 synthesis. Disappointingly, only 2 of 6 studies detailing the phenotypes PCSK1 mutant probands assess post-prandial GLP-1 secretory responses and report conflicting results; whilst an oral glucose load (OGTT) yields significantly reduced GLP-1 response in three child probands compared to age matched controls, post-prandial responses in a 40-year-old proband match those of healthy age-matched controls. One interpretation of such findings may be that whilst other PCs may compensate for lacking PC1/3 to allow for GLP-1 synthesis in response to mixed nutrient secretagogues, PC1/3 is necessary and essential for GLP-1 synthesis in response to its most potent secretagogue, glucose. An alternative interpretation comes from observations that GLP-1 secretion following OGTT in the 3 child probands studied by Bandsma *et al*<sup>[146]</sup> seem to show an age dependent impairment improving with increasing age. Following follows reports by Parker *et al*<sup>[147]</sup> who observed that the pattern of endocrinopathy in probands with PCSK1 mutant monogenic obesity change with age, perhaps GLP-1 secretion too may show an age-dependent alteration, potentially compensated for over time. One way to test this hypothesis would be to histologically examine the enteroendocrine expression of GLP-1 in adult PCSK1-mutant probands; enteroendocrine expression of GLP-1 is significantly reduced compared to control in children with PCSK1 monogenic obesity<sup>[146]</sup>, if indeed the normal post-prandial GLP-1 responses seen in adulthood are a reflection of the activation of redundant PC activity in intestinal cells up regulation of enteroendocrine GLP-1 expression would be observed. Though the cause of the hyperphagic obesity in PCSK1 mutant human monogenic obesity remains ill defined,

monogenic obesity implicates a role for deregulated GLP-1 signalling in the development of the obesity phenotype.

### **Polygenic obesity and Genome Wide Association**

**Studies:** Monogenic obesity is a rare form of clinical obesity, accounting for less than 1% of total cases of obesity worldwide. The obesity epidemic of the past 10-50 years has been largely attributed to environmental and societal changes facilitating a positive energy balance; "the obesogenic environment"<sup>[149,150]</sup>. However evidence from adoption, twin and family studies suggest the genetic contribution to BMI ranges between 60% and 84%<sup>[151]</sup>. As such, the current obesity epidemic may be defined as the interaction between a genetic predisposition and the "obesogenic environment"<sup>[149,150,152-154]</sup>. Genome wide association studies have identified 119 independent gene loci implicated as risk factors toward "common" obesity<sup>[155,156]</sup>, as such today's obesity epidemic may be referred to as a polygenic obesity. One such susceptibility gene is PCSK1 encoding the enzyme PC1/3 involved in the proteolytic processing of proglucagon, to yield, amongst other peptides, GLP-1.

Single-nucleotide polymorphisms (SNPs) at three independent *PCSK1* loci have been consistently linked to an increased risk of obesity<sup>[157-161]</sup>. Though it is unclear how these minor alleles predispose to obesity, *in vitro* studies suggest that the encoded PC1/3 variants may not be as enzymatically active or physiologically available as the common form, potentially resulting in a partial PC1/3 deficiency. Decreased GLP-1 synthesis secondary to reduced proglucagon processing by PC1/3 in enteroendocrine L cells may therefore be the mechanism by which identified PCSK1 SNPs confer an increased risk toward the obesity phenotype.

### **Intestinal neuroendocrine gene expression:**

Neuroendocrine signals from the gut play an important role in the physiological control of energy balance. Findings from a recent study by Ritze *et al.*<sup>[162]</sup> studying the gene expression of several proteins in the intestinal neuroendocrine network go some way to suggest intestinal GLP-1 expression and/or function may be altered in obesity. Though GLP-1 was not directly tested in the study, the anorectic neuropeptide PYY shown to co-localise and be co-secreted with GLP-1 in enteroendocrine cells<sup>[163]</sup> was tested. Taking PYY levels as proxy measures of GLP-1, Ritze *et al.*<sup>[162]</sup> report significant correlations between GLP-1 with the GLP-1R in non-obese subjects (suggesting physiological ligand-receptor signalling), a correlation lost in obese subjects and replaced by correlations with the orexigen ghrelin ( $P < 0.01$ ). Ritze *et al.*<sup>[162]</sup> also observed correlations between the long-term satiety signal leptin and GLP-1R in obese subjects not seen in their lean counterparts.

A recent *in vitro* study on human L cells has shown that ghrelin is a positive modulator of GLP-1 release<sup>[164]</sup>. Ghrelin levels have also been reported to be reduced in humans with obesity<sup>[165,166]</sup>. The correlations between

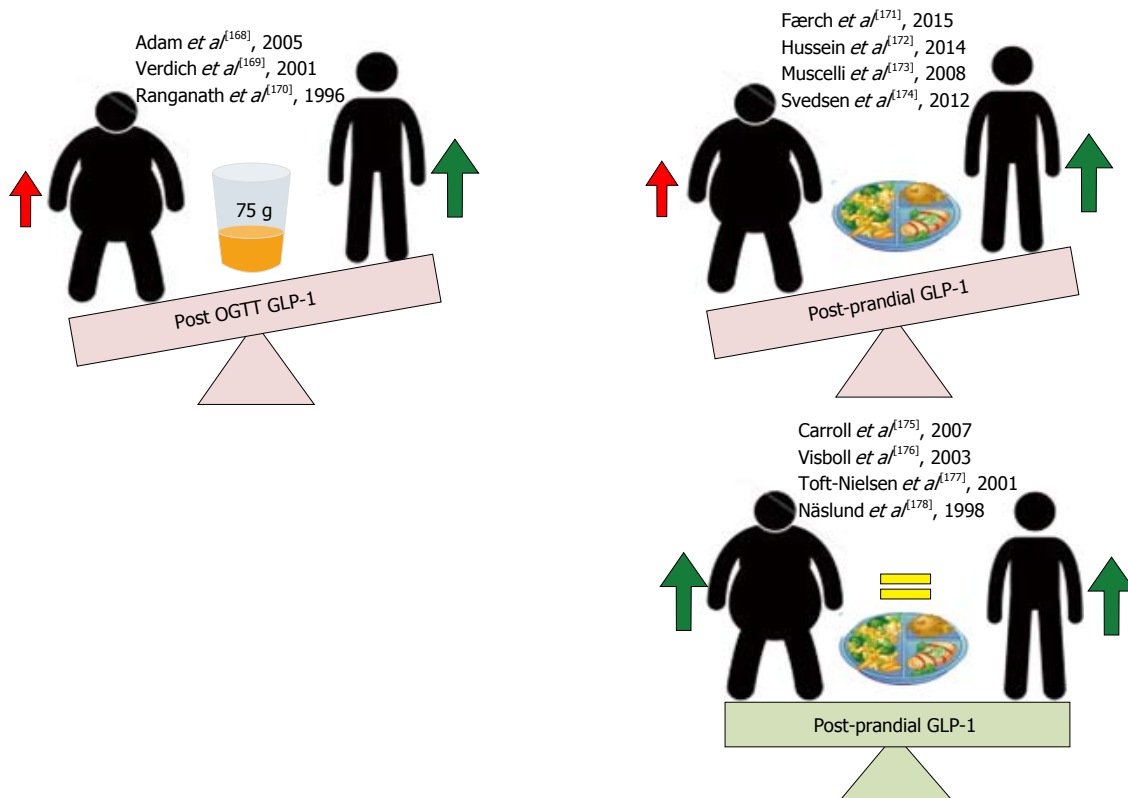
intestinal PYY (GLP-1) and ghrelin reported in obese subjects suggests that ghrelin decreases in obesity coincide with decreased GLP-1 levels, the latter potentially antagonising the anorexigenic effects of the former and may explain the difficulty to attain and maintain weight loss observed by many obese patients. Intestinal GLP-1 signalling has been suggested to promote small-intestinal motility in humans<sup>[167]</sup> and preclinically, central administration of leptin has been shown to increase the satiating effect of GLP-1, possibly through enhancing GLP-1R signalling. Correlations between the leptin and GLP-1R in obese subjects may therefore reflect a leptin-mediated enhancement of intestinal GLP-1/GLP-1R increasing intestinal motility to promote increased gastric emptying and reduced gastric mechanoreceptor activation in response to a given meal; the resultant decrease in anorexigenic signalling potentially explaining the persistent hyperphagia seen in obesity despite an overall positive energy balance.

### **Clinical studies in polygenic obesity**

Interventional and observational clinical evidence suggests that malfunctioning of GLP-1 contributes to the development and/or maintenance of the obesity phenotype, rationalizing the use of GLP-1 analogues as novel therapeutic agents in the medical management of obesity.

### **Post meal and oral glucose GLP-1 secretory responses:**

A number of clinical studies have assessed the effect of physiological GLP-1 secretion responses in obese and lean subjects following an oral 75 g glucose load (OGTT) or post-prandial following a balanced meal. Where an OGTT consistently demonstrates a reduced GLP-1 secretion in obese subjects compared to their lean control post-prandial GLP-1 responses are conflicting; some observing significant reductions and others no change<sup>[168-178]</sup> in obese subjects when compared to their lean counterparts (Figure 8). That oral glucose, the most powerful GLP-1 secretagogue consistently demonstrates reduced GLP-1 responses in obese subjects may suggest that the impaired GLP-1 response observed are secondary to a reduced L-cell glucose sensing capacity in obesity. Support for such a postulate comes from findings by Ranganath *et al.*<sup>[170]</sup> who demonstrate that whilst GLP-1 secretion to an oral fat load remains intact, GLP-1 secretion in response to an oral carbohydrate load is decreased in obesity. However, reports by Adam *et al.*<sup>[168]</sup> that demonstrate reductions in GLP-1 response in obesity to a balance meal with retained responses to an oral carbohydrate load challenge such an interpretation. Interestingly however, in their observational study, Adam *et al.*<sup>[168]</sup> also demonstrate that whilst carbohydrates stimulate post-prandial GLP-1 release similarly in obese and non-obese subjects, these rises are positively correlated with increased satiety only in lean subjects and put forward an alternate hypothesis that rather than impaired GLP-1 secretion, downstream receptor resistance may be the root of GLP-1 dyshomeostasis in



**Figure 8** Effects of obesity on glucagon-like peptide 1 responses post oral glucose load and post-prandial. Obese subjects consistently demonstrate reduced glucagon-like peptide 1 (GLP-1) secretory responses following a 75-g oral glucose load compared to lean controls. Post-prandial GLP-1 secretory responses in obese subjects are conflicting, with some studies observing significant reductions and others observing no change<sup>[168-178]</sup> when compared to lean controls.

obesity. That deranged GLP-1 signalling is observed only in obese subjects suggests that obesity induces changes in functional GLP-1 signalling that through resultant reductions of signalling at central and peripherally located receptors (GLP-1) may facilitate the maintenance of the obesity phenotype. Pharmacologically targeting GLP-1 to restore physiological signalling may therefore be an efficacious method to prevent the propagation of, and potentially reverse weight gain in obesity.

Evidence from clinical studies suggests that weight gain induces alterations in functional GLP-1 signalling that facilitates and propagates the obesity phenotype. Though the mechanisms of reduced functional post-prandial GLP-1 signalling in obesity remain to be defined, clinical evidence implicates a role for interactions between GLP-1 and the long-term satiety signals insulin and leptin, and short-term orexigen ghrelin. The most extensively studied of post-prandial GLP-1's physiological roles is as a positive modulator of pancreatic  $\beta$ -cell insulin secretion<sup>[123]</sup>. Hyperinsulinaemia is positively associated increased BMI in individuals with normal glucose tolerance and increased BMI and increasing glucose intolerance have been shown to independently and additively impair GLP-1 secretion<sup>[171-173,175,179]</sup>. The chronic hyperinsulinaemia positively associated with increasing levels of obesity therefore, may acting as a negative feedback signal to inhibit physiological post-prandial GLP-1 release observed in obese subjects when compared with healthy lean control<sup>[171-173]</sup>. The long term

adiposity signal leptin acts as a satiety signal governing long term energy balance and clinically, increased BMI has been shown to be positively correlated with fasted leptin, however obese subjects are thought to be resistant to leptin's effects<sup>[180,181]</sup>. *In vitro* studies of human intestinal L-cells have shown that leptin acts a GLP-1 secretagogue and go some way to suggest that the leptin resistance associated with obesity may account for the decreased post-prandial GLP-1 secretion observed in obese humans<sup>[168-170,182]</sup>. Ghrelin is the only orexigenic gut derived hormone<sup>[183]</sup>; released pre-prandial, ghrelin promotes meal initiation and increases food intake, and complex reciprocal interactions exist between GLP-1 and ghrelin that have implications for obesity pathophysiology. Preclinically, physiological ghrelin signalling has been shown to enhance post-prandial GLP-1 release<sup>[164]</sup>, clinical obesity has however been associated with reductions in fasting ghrelin levels that may contribute to the reduced post-prandial GLP-1 release observed<sup>[168-170]</sup>. Conversely, clinical data exists to suggest that suppression of late post-prandial rises in ghrelin is one mechanism by which GLP-1 exerts its anorexigenic effect<sup>[126]</sup>; reduced post-prandial GLP-1 secretion in obesity potentially explaining the attenuated decreases of post-prandial serum ghrelin observed in this cohort<sup>[168-170,184,185]</sup> (Figure 9).

Together, evidence exists to suggest that the hyperinsulinaemia, leptin resistance and impaired ghrelin secretion occurring secondary to obesity cause functional deficits in GLP-1 signalling; the resultant reductions in



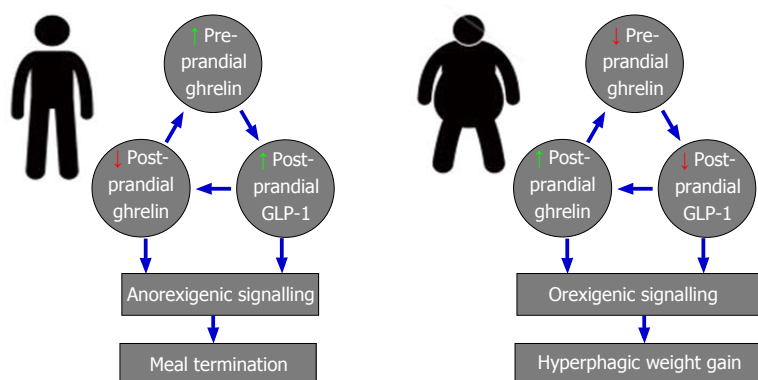


Figure 9 Physiological and pathophysiological interactions between orexigenic ghrelin and anorexigenic glucagon-like peptide 1 signalling in lean and obese individuals. GLP-1: Glucagon-like peptide 1.

GLP-1 mediated anorexigenic signalling facilitating post-meal hyperphagia, weight gain and thus perhaps the obesity phenotype. Pharmacologically targeting GLP-1 to restore homeostatic signalling may therefore be an efficacious method to prevent the propagation of, and potentially reverse the weight gain in obesity.

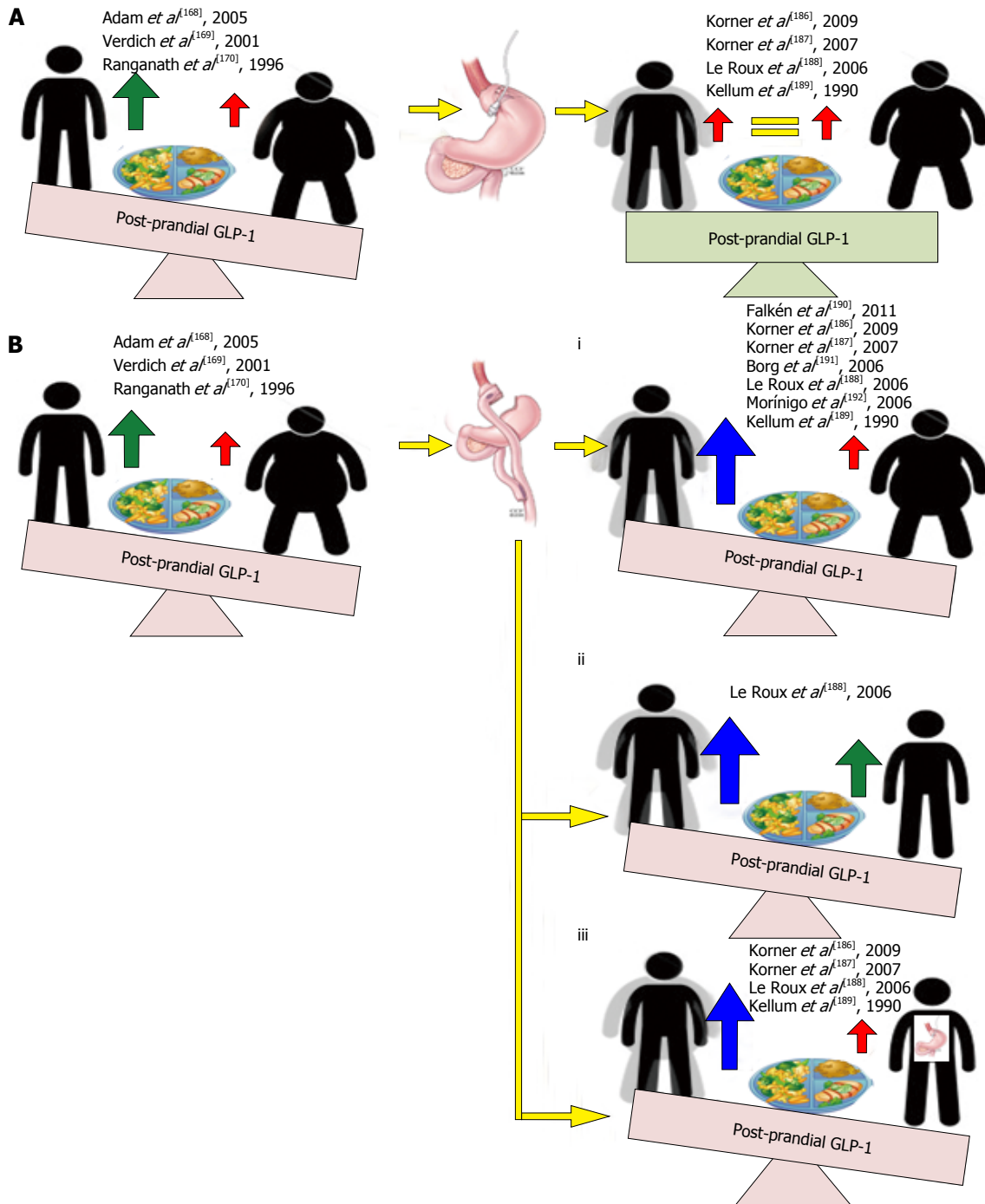
**GLP-1 secretion post RYGB:** Weight losses following bariatric surgery, pharmacotherapy or diet and lifestyle modification are all associated with decreases in circulating leptin and improved insulin sensitivity. The resultant reductions in anorexigenic signalling potentially facilitating weight gain and may explain the difficulty obese subjects have in attaining and maintaining weight loss. Bariatric surgery remains the most effective treatment modality for morbid obesity, with a meta-analysis reporting the Roux-en-y gastric bypass (RYGB) to produce a greater and more sustained weight loss than currently available pharmacotherapeutics, diet and lifestyle interventions or other bariatric options. Prospective studies assessing the effects of RYGB on post-prandial GLP-1 responses in non-diabetic obese patients consistently report statistically significant increases in post-prandial GLP-1 when compared to the pre-operative state, following equivalent weight losses with GB<sup>[186-193]</sup> and when compared with healthy lean control<sup>[188]</sup> (Figure 10). This post-operative supraphysiological GLP-1 secretory response therefore may explain the greater short- and long-term weight loss efficacy achieved with this treatment modality.

Evidence from clinical studies implicate the supra-physiological<sup>[188]</sup> post-prandial GLP-1 responses achieved following RYGB in the superior weight loss efficacy of this treatment modality. Though the mechanisms by which RYGB may induce increases in GLP-1 secretion remain poorly understood, clinical studies implicate a role for altered gut mechanics and L cell resensitisation.

That increases in post-prandial GLP-1 responses following RYGB are observed as early as 3 d post-operatively<sup>[190]</sup> suggest physical changes associated with RYGB, rather than gene-mediated up-regulations GLP-1 synthesis play a role in the increased GLP-1 secretory

responses observed. Where both RYGB and GB induce weight loss through volume restriction, the former also redirects nutrient flow from the upper stomach directly into the distal jejunum. The exaggerated GLP-1 response following RYGB likely secondary to the increased glucose load delivered to the distal small intestine where L-cells are more densely populated. Such a concept is supported by the observed reductions in foregut and increases in hindgut hormones following RYGB and the hyperplasia of GLP-1 containing ileal cells in biopsy samples of obese humans after bypass<sup>[194-196]</sup>.

RYGB induces a weight loss greater time-for-time and reaches a plateau more successfully maintained when compared with weight loss following GB<sup>[186-189]</sup>. Post-prandial GLP-1 responses following RYGB are also significantly greater than those following GB (that show no change from pre-operative levels<sup>[186,190,191]</sup>) however this response does not plateau but instead shows a tendency to increase with time past surgery. A relationship between the exponentially increasing post-prandial GLP-1 response and greater weight loss maintenance achieved post-RYGB may be explained by findings observed Kellum *et al.*<sup>[189]</sup> who report that at 1 year post RYGB, alongside significantly greater achieved and maintained weight loss when compared to GB, GLP-1 responses were significantly increased in response to a carbohydrate meal in subjects post RYGB; a response positively associated with amount of weight lost. With derangements in L-cell carbohydrate sensing implicated in the pathophysiology of human obesity (see 5.1.1B, 5.2.1B), and with no alterations in response to a protein-fat meal observed following weight loss with RYGB and no altered response to either meal following weight loss with GB, findings suggest that weight loss following RYGB may be associated with a restoration of L cell sensitivity to the most potent GLP-1 secretagogue; a resensitisation that may occur proportionately to the amount of weight lost, the feed forward effect of weight loss on increased GLP-1 secretion resulting in supraphysiological GLP-1 signalling with the potential to antagonize the increased orexigenic drives of decreased leptin and insulin signalling associated with weight loss.



**Figure 10** Effects of weight loss induced by gastric banding and Roux-en-Y gastric bypass on post-prandial glucagon-like peptide 1 secretion. Weight loss following gastric banding induces no changes in post-prandial glucagon-like peptide 1 (GLP-1) levels from pre-operative levels (A). All 7 studies assessing the effects of post-prandial GLP-1 secretion following weight loss with RYGB show significantly increased responses compared with pre-surgery responses and healthy obese controls (Bi), healthy lean controls (Bii) and following weight losses following gastric banding (Biii).

Such a concept may explain the long-term weight loss efficacy associated with RYGB.

Together, evidence exists to suggest that the supraphysiological<sup>[188]</sup> upregulation in GLP-1 signalling seen following RYGB contributes to the superior short and long term weight loss efficacy observed with this treatment modality. Not only do findings go some way to suggest a role for impaired GLP-1 signalling in the pathophysiology of human obesity, findings support the potential for pharmacological mimicry of this supraphysiological GLP-1

secretion as a minimally invasive, thus risk reducing and cost-effective alternative of achieving and maintaining similarly significant weight loss in obese subjects in the clinic.

**Functional neuroimaging and self-assessments of appetite:** fMRI studies have provided evidence *in vivo* to suggest that central nervous system responses in brain regions involved in rewards processing are altered in obese individuals; reduced brain activity in response to the

consumption of, and increased activity in response to the anticipation of palatable food consistently observed when compared with healthy lean controls<sup>[197-199]</sup>. Interestingly, GLP-1 agonism reverses these functional brain changes to match those of lean control with associated reductions in *ad libitum* food intake<sup>[199]</sup>, an effect prevented by pre-treatment with a GLP-1 antagonist<sup>[200]</sup> (Figure 11). Together, findings suggests that obesity induced decreases in functional GLP-1 signalling contribute to altered rewards processing in obesity to facilitate hyperphagic weight gain despite an overall positive energy balance.

Further support for a role for GLP-1 and altered rewards processing in obesity pathophysiology comes from self-assessments of appetite. Subjectively assessed emotional eating scores have been defined as hedonic markers of appetite that display strong positive associations with the degree of human obesity, and, in obese subjects, relate to the extent to which GLP-1 receptor activation in brain regions involved in rewards processing are reduced<sup>[201-203]</sup>. Together, findings suggests that obesity induced decreases in functional GLP-1 signalling creates a feed-forward loop of hyperphagic weight gain despite an overall positive energy balance, an effect perhaps secondary to a GLP-1 deficit mediated skew toward hedonic and away from homeostatic controls of food intake. Together, findings from fMRI and subjective appetite assessment scores implicate obesity-associated reductions in functional GLP-1/GLP-1R signalling in the pathophysiology of hyperphagic weight gain in obesity. As such, findings support the role of the GLP-1R as a novel therapeutic target in the medical management of obesity, providing rationale for the use of liraglutide 3 mg in the pharmacotherapy of obesity in the clinic.

## A ROLE FOR GLP-1 IN THE PHARMACOTHERAPY OF CLINICAL OBESITY

The balance between drug efficacy and cost determines the selection of a pharmacological agent for use in the medical management of any disease; greater understanding of underlying disease pathophysiology facilitating the development of targeted therapeutics with the potential for greater efficacy. Several lines of clinical evidence implicate a role for altered GLP-1 function in the pathophysiology of human obesity and a number of recent clinical trials have validated the clinical efficacy of long-term once daily SC 3 mg liraglutide (Saxenda) as an adjunct to calorie-restriction and exercise counselling in obese and overweight individuals with at least one weight related comorbidity. Significant improvements in clinical outcome measures such as body weight, anthropometric and cardiometabolic parameters, and indices of glucose tolerance have been observed and recently reviewed elsewhere<sup>[204]</sup>. Though March 2015 saw the EMA grant marketing authorization for 3 mg liraglutide as a weight-management agent in all 28 EU states<sup>[45]</sup>, cost-benefit of funding treatment on the NHS undoubtedly contributes

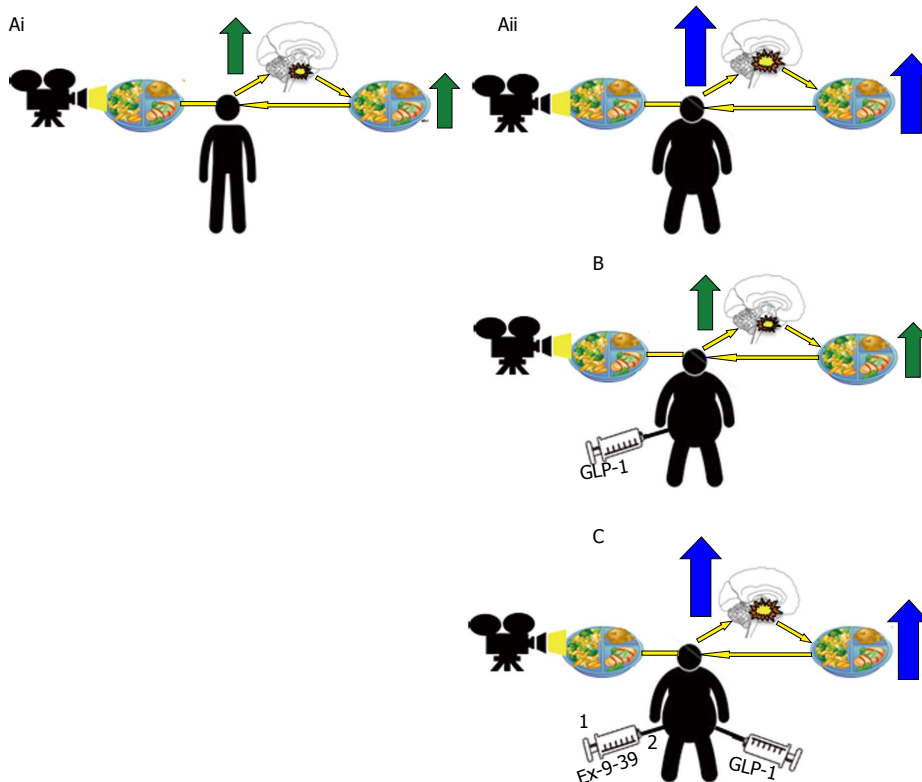
to the uncertainty of launch plans in the United Kingdom at present<sup>[46]</sup>.

### Weight loss efficacy of GLP-1 analogues

**Evidence:** One phase II (NCT00422058)<sup>[43]</sup>, and a number of phase III multi-national double-blinded randomized control trials conducted in non-diabetic obese adults (NCT00480909)<sup>[42]</sup>, overweight adults with at least one weight related co-morbidity (SCALE Obesity and Pre-diabetes, and SCALE Maintenance<sup>[205,206]</sup>), non-diabetic obese adults with obstructive sleep apnoea (OSA) (SCALE OSA<sup>[41]</sup>) and obese adults with T2DM (SCALE diabetes<sup>[207]</sup>) have established the efficacy of once daily 3 mg SC liraglutide as an adjunct to an energy-deficient low-calorie diet and physical activity counselling for weight management in this cohort. Results from the first study; a 20-wk phase II trial in non-diabetic obese subjects showed that weight loss with liraglutide is dose-dependent up to 3.0 mg once daily<sup>[42,206]</sup>. Significantly more liraglutide 3 mg/d recipients than placebo or orlistat recipients achieved a 5% or 10% reduction of body weight at 20 wk. In a 2-year phase III extension of the same study<sup>[42]</sup>, double-blind treatment (liraglutide 1.2-3 mg/d) was continued until week 52, after which all liraglutide (< 2.4 mg/d) and placebo recipients were switched to liraglutide 2.4 mg, then 3.0 mg (week 70-96) based on 20-wk and 1-year results respectively (Figure 12) that indicated this was the optimal dosage. At 2 years, mean bodyweight reductions in those randomized to liraglutide were significantly greater than pancreatic lipase inhibitor orlistat, the only alternative licenced weight loss agent in the United Kingdom. Results from the SCALE maintenance and SCALE obesity and prediabetes<sup>[205,206]</sup> trials report similarly significant reductions in bodyweight in subjects randomized to 3 mg liraglutide when compared with placebo at 56 wk ( $P < 0.0001$ ) alongside increased 5% and 10% responder rates. Findings are supported by results of the 32 wk SCALE Sleep Apnoea trial<sup>[41]</sup> in obese non-diabetic subjects with moderate to severe OSA and in the 56-68 wk SCALE Diabetes<sup>[207]</sup> trial in obese subjects with T2DM (Figure 13). With even modest losses of 5%-10% of total body weight associated with reduced risk of comorbidities in obese individuals<sup>[8-10]</sup>, findings provide rationale for the licensing and funding of 3 mg liraglutide as an adjunct to lifestyle alteration as the first line anti-obesity pharmaceutical agent for weight management in obese and comorbid overweight adults in the United Kingdom.

### Interpretations - obesity pathophysiology:

Excessive consumption of palatable food can trigger neuroadaptive responses in brain reward circuits similar to that of alcohol and drugs of abuse<sup>[208]</sup> and clinical studies provide evidence to suggest that human obesity is associated with altered rewards processing mediated in part by altered GLP-1 function that may render the hyperphagia of obesity the manifestation of a "food addiction". Whilst 3 mg liraglutide has been shown to induce weight loss in man by reductions appetite pre-



**Figure 11** Altered central nervous system responses in brain regions involved in rewards in obese subjects is reversed upon glucagon-like peptide 1 receptor activation. Central nervous system activity in regions involved in rewards processing show increased responses in obese subjects when exposed to food related images (Aii) associated with increased ad libitum food consumption when compared to healthy lean control (Ai). A response reversed upon administration of a GLP-1 analogue with concomitant reductions in ad libitum food intake (B), this effect antagonized by pre-treatment with GLP-1 antagonist Extendin 9-39 (EX9-39) (C). GLP-1: Glucagon-like peptide 1.

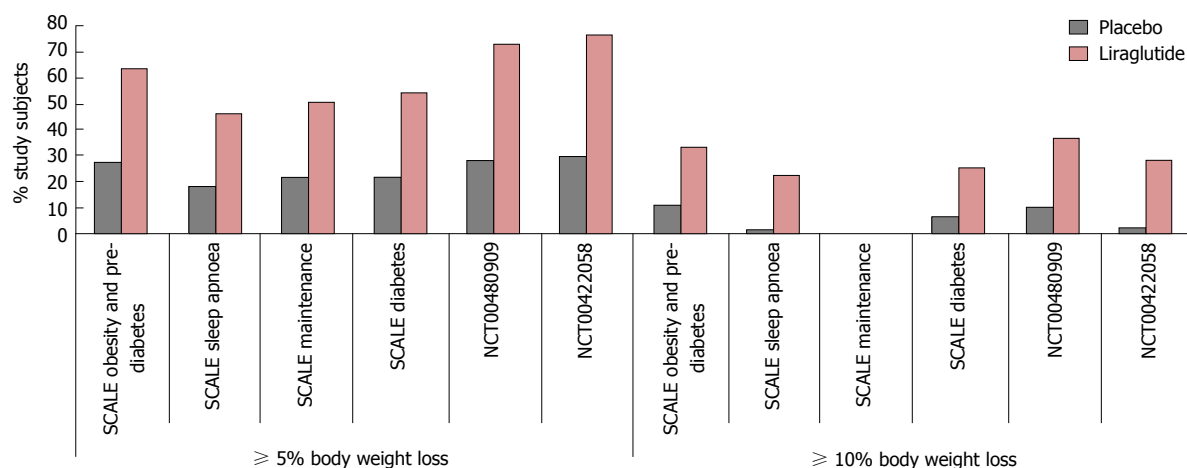
clinically, liraglutide attenuates the reinforcing properties of alcohol *in vivo*<sup>[209,210]</sup>. As such, perhaps GLP-1 agonism may attenuate produce its weight loss effects in part, by attenuating the negative reinforcement of hyperphagia in obesity. Interestingly, though all aforementioned trials<sup>[41-43,205-207]</sup> advise participants to restrict food consumption throughout the treatment period, adherence rates are not reported. If indeed GLP-1 agonism induces its weight loss effects by modulating food related rewards that potentially reverses the negative reinforcement of hyperphagia in obesity, an increased adherence to caloric restriction would be expected. It would be interesting to see if this were the case.

**Interpretations - cost-benefit of 3 mg liraglutide as an anti-obesity agent on the NHS:** Follow-up period (FUP) assessments in the SCALE Maintenance, and SCALE Diabetes<sup>[205-207]</sup> trials suggest that weight loss with 3 mg liraglutide is treatment dependent; weight gains in excess to those seen in placebo control and subjects re-randomized to treatment observed in liraglutide treated participants upon treatment cessation (Figure 12).

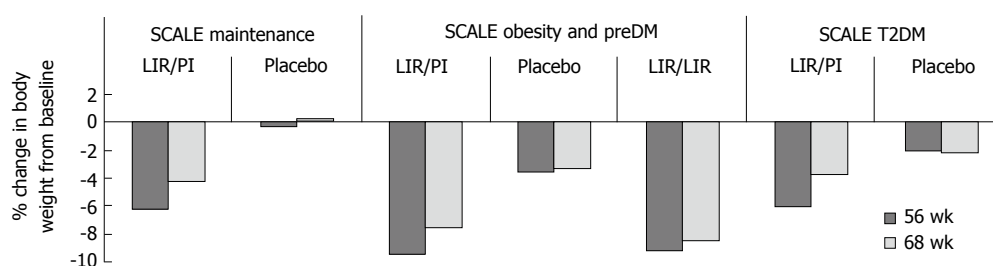
Though reductions in bodyweight have been shown to significantly improve health outcomes and thereby reduce healthcare costs long term<sup>[211]</sup>, in 2013, 24.9% of the United Kingdom adult population were classed obese and 90% of the £2.7 million T2DM adults in the United Kingdom were overweight. Following FDA and

EMA approval criteria, all these individuals are potential candidates for treatment with liraglutide 3 mg. Costing in excess of \$1000 per patient a month, and with prevalence of obesity and overweight predicted to rise (introduction), prolonged treatment seems unsustainable. A potential solution comes from longitudinal observations from Astrup *et al*<sup>[43]</sup> who observed that subjects randomized to liraglutide 3 mg achieve maximal rates of weight loss in the initial 0-20 wk treatment period with a tendency toward weight gain beyond 36 wk<sup>[42,43]</sup>. Findings suggest that whilst initially treatment with a GLP-1 analogue may compensate for functional deficits in obesity, treatment beyond 20 wk may be associated with the development of treatment resistance, most apparent 36 wk from initiation (Figure 14). Based on this, perhaps treatment with liraglutide 3 mg should be prescribed for 20 to a maximum of 36 wk alongside behavioural therapies promoting lifestyle changes and developing strategies to combat the addiction driven hyperphagia implicated in obesity pathophysiology (GLP-1 secretion post RYGB); combining behavioural therapies in the initial 20 wk of drug induction where weight loss is most pronounced may act as a positive reinforcer of sustained behavioural change facilitating continuation of these behaviours. This approach, integrating the psychosocial empowerment associated with patient self-management of chronic illness, alongside cost benefits associated with limited in-treatment period seems an





**Figure 12** Significantly greater 5% and 10% weight loss achieved following 3 mg subcutaneous liraglutide compared to placebo and orlistat. Five percent and 10% responder rates in NCT00480909 reported at 1 year (see text).



**Figure 13** Body weight changes with liraglutide are treatment dependent. Following the 56-wk treatment period a 12-wk follow-up (FUP) was conducted in SCALE maintenance, SCALE obesity and pre-diabetes and SCALE diabetes trials. Twelve weeks FUP was an off-treatment period in SCALE Maintenance and SCALE diabetes. In SCALE Obesity and pre-diabetes FUP period involved a re-randomisation to either 3 mg liraglutide (LIR/LIR) or placebo (LIR/PI) and though weight gain occurred in all three groups weight gain was significantly higher following cessation of liraglutide. T2DM: Type 2 diabetes mellitus.

attractive one, especially if sustainable long-term weight losses with resultant reductions in the socioeconomic impacts of weight-related comorbidities can be achieved.

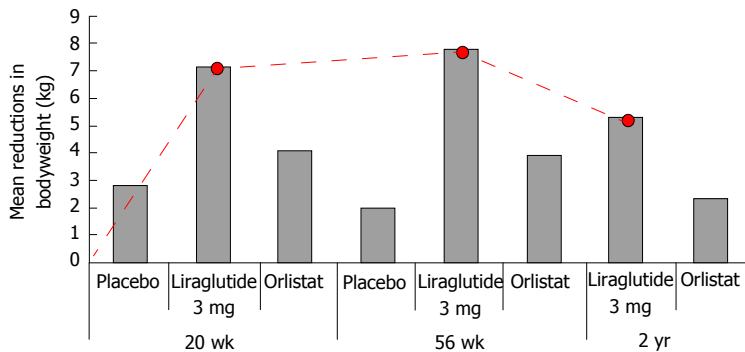
### Glucose tolerance

**Evidence:** Clinical studies in obese subjects with pre-diabetes consistently demonstrate a greater reversion to normal glycaemic control following treatment with liraglutide 3 mg coinciding reduced T2DM incidence<sup>[42,206,212]</sup>.

**Interpretations - obesity pathophysiology:** Being overweight or obese is the main modifiable risk factor for T2DM and increasing BMI is positively associated with hyperinsulinaemia even in those with normal glycaemic control<sup>[175,179,213]</sup> suggesting a common pathophysiology to both conditions. That increased BMI and impaired glucose tolerance have been shown to independently and additively impair GLP-1 secretory responses following an OGTT<sup>[171-173]</sup> suggests that this common pathophysiology may lie in a functional deficit of GLP-1. Support for the existence of a common pathophysiology between obesity and T2DM comes from observations that treatment with the insulin sensitizer metformin (currently the first line pharmacotherapeutic agent in the management of T2DM) upregulates GLP-1 secretory response following

an OGTT, the restoration of physiological anorexigenic and incretin effects perhaps explaining the weight loss, and insulin sensitising properties of the drug seen in the clinic respectively<sup>[214,215]</sup>.

**Interpretations:** Cost-benefit of 3 mg liraglutide as an anti-obesity agent on the NHS: Being overweight or obese is the main modifiable risk factor for T2DM<sup>[213]</sup> and T2DM is one of the major indirect financial burdens of obesity and overweight. Treating T2DM and its complications alone current costs the NHS £8.8 billion a year with indirect costs estimated at £13 billion<sup>[216]</sup>. With the incidence of obesity projected to rise (introduction), so too can be expected the incidence of T2DM, the management of which therefore, may become unsustainable on the NHS. Though costly, treatment with liraglutide 3 mg is associated with reductions in the rate of development of T2DM in overweight and obese subjects<sup>[42,206,212]</sup> and goes some way to suggest that treatment may reduce both direct burdens of obesity and overweight and the large indirect burden posed by new incidences of T2DM. However, weight loss in itself is associated with an improvement in glycaemic control. It may be argued therefore, that true cost-benefit of funding liraglutide 3 mg on the rationale of T2DM prevention in overweight and obese



**Figure 14** Liraglutide 3 mg shows greater, dose dependent, reductions in body weight compared to placebo and orlistat with maximal effect between 0-20 wk of treatment. Red line dotted trace shows patterns of weight loss from baseline in those subjects treated with 3 mg liraglutide. Greatest rate of weight loss was observed between 0-20 wk, reducing from 20-36 with a tendency toward weight gain from 36 wk and beyond.

subjects exists only if the improved glucose tolerance achieved following a given weight loss with liraglutide 3 mg exceeds those attained following other, arguably cheaper treatment modalities. Calculating correlation coefficients between percentage weight lost and percentage changes in glucose tolerance in non-diabetic obese subjects randomized to liraglutide 3 mg compared to those administered orlistat or placebo<sup>[41-43,205,206]</sup> in aforementioned phase III trials may be one way in which this could be assessed. Figure 15 interprets the possible findings of such a test.

If indeed pharmacological GLP-1 agonism improves glucose tolerance independent of weight lost, the potential curb in prevalence, thus socio-economic burden of T2DM achieved with treatment provides a second rationale for the licensing and funding of liraglutide 3 mg as an adjunct to lifestyle alteration as the first line anti-obesity agent for weight management in obese and co-morbid overweight individuals in the United Kingdom.

### Adverse drug events

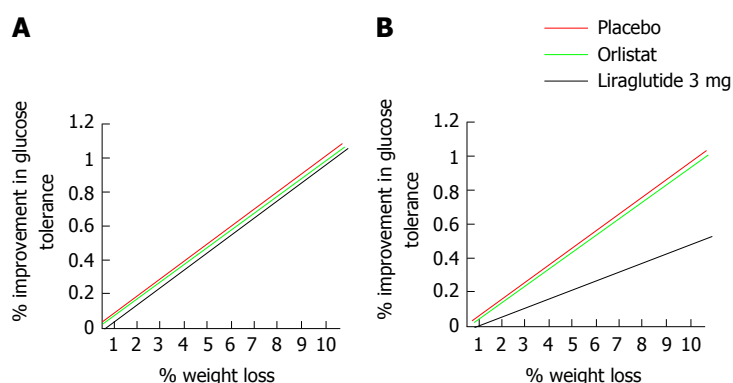
**Evidence - in-treatment tolerability:** Whilst evidence exists to suggest GLP-1 agonism may be a targeted agent with long term cost-benefit for use in the medical management of obesity, tolerability and safety are important considerations in determining the choice of any pharmaceutical, especially in the management of chronic disease. The safety and efficacy of liraglutide 3 mg has been evaluated in 5 phase III double-blinded placebo controlled trials comprising 3384 overweight or obese subjects receiving liraglutide 3 mg and 1941 placebo controls for a treatment periods of 32, 52 and 56 wk<sup>[42,42,205-207]</sup>. In a pooled analysis of the 5 aforementioned trials, liraglutide 3 mg in obese and overweight subjects was generally well tolerated, with most adverse drug events gastrointestinal in nature, transient and of mild to moderate intensity<sup>[42,43,205-207]</sup>. However, 9.8% of liraglutide and 4.3% of placebo recipients discontinued treatment because of an adverse event<sup>[217]</sup>. Figure 16 details adverse reactions occurring with a higher incidence to placebo with an incidence of  $\geq 10\%$  in liraglutide 3 mg recipients, stratified by system. Of interest, 0.6% of subjects receiving liraglutide

3 mg experienced increases in mean heart rate (an average baseline increase of 2.5 beats/min) compared to 0.1% of placebo recipients<sup>[218]</sup>. Potentially a manifestation of GLP-1 induced increases in SNS activity (potential effectors of GLP-1s negative energy balance effects: Central controllers) contributing to GLP-1 induced weight loss *via* increases energy expenditure, elsewhere tachycardia associated with 3 mg liraglutide treatment in non-diabetic obese subjects yields no associated increases in 24 h energy expenditure<sup>[209]</sup>. Thus, whilst the clinical significance of this finding remains to be determined, observations may warrant more intense monitoring in patients with pre-existing cardiovascular disease.

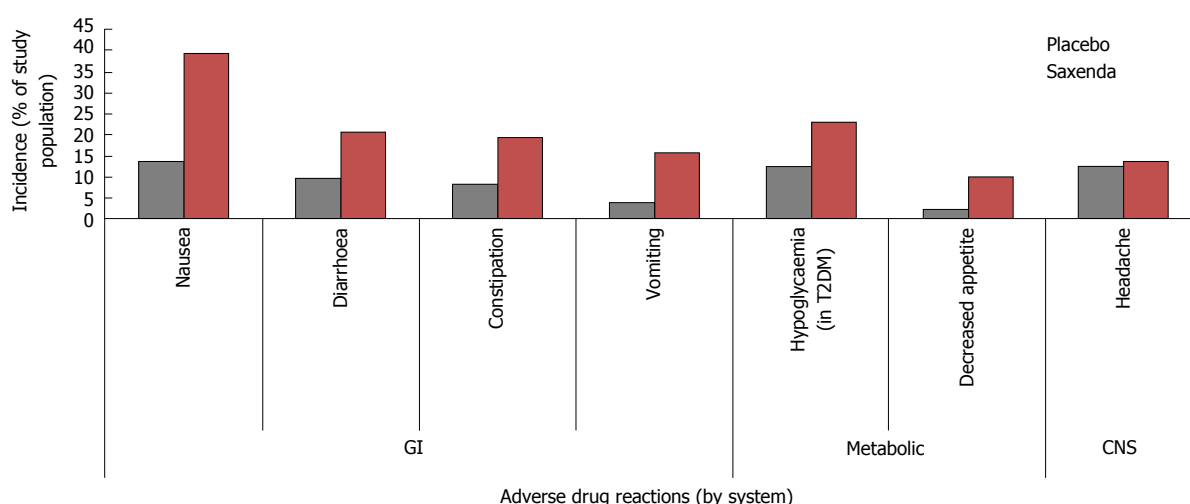
### Interpretations - long term risk-benefit of liraglutide 3 mg as an anti-obesity agent on the NHS:

Though generally well tolerated in the acute setting, safety concerns have been raised regarding the potential risk of pancreatitis and pancreatic and thyroid cancer with long-term use of GLP-1 analogues<sup>[219,220]</sup>. Confirmed cases of acute pancreatitis and papillary thyroid carcinoma were reported in 0.3% of liraglutide 3 mg treated compared to 0.2% of placebo treated participants, however the relative rarity of events means the relationship between treatment with disease incidence and severity remains to be defined. Ongoing clinical experience and thorough post-marketing surveillance should help clarify any such associations and also identify other potential adverse drug events. To this end, episodes of acute renal failure and medullary thyroid carcinoma (not observed during in-treatment and FUP period assessments<sup>[42,43,205-207]</sup>) have been reported in the post-marketing period, though again, insufficient data exists to establish or exclude a causal relationship. Figure 17 details other potentially serious medical conditions observed in during in-treatment and FUP assessments<sup>[42,43,205-207]</sup>.

Though potentially associated with serious long-term adverse effects, the rarity of incidence and lack of causal relationship mean that current knowledge supports benefit over risk, supporting the licensing and funding of liraglutide 3 mg as the first line anti-obesity pharmaceutical agent for weight management in obese and overweight adults with at-least one weight related



**Figure 15 Theoretic model to assess glucose tolerance as a factor of weight.** Liraglutide 3 mg produces increased weight loss and increased improvements in glucose control compared to both placebo and control in non-diabetic obese and overweight subjects<sup>[41-43,205,206]</sup>. If the greater glucose sensitivity seen following treatment with liraglutide 3 mg is secondary to the greater weight loss achieved, the relationship between a given weight reduction and the percentage improvement in glucose tolerance should be the same in all treatment groups (A). If however, the relationship between a given weight loss and change in glucose tolerance is less strong (B) following treatment with liraglutide, findings would suggest that mechanisms beyond weight loss contribute to the greater improvements of glucose tolerance seen following GLP-1 agonism. One mechanism may be *via* activation of GLP-1R in the pancreas, where endogenous GLP-1 signalling has a well established incretin effect. GLP-1: Glucagon-like peptide 1.



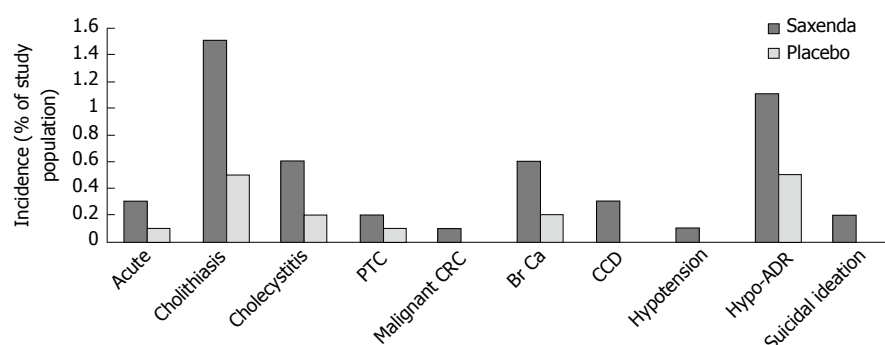
**Figure 16 Adverse drug reactions reported in  $\geq 10\%$  of liraglutide 3 mg recipients with a higher incidence than placebo stratified by system.** GI: Dyspepsia, abdominal pain, dry mouth, gastritis, gastroesophageal reflux disease, flatulence, eructation and abdominal distension were also more prevalent in liraglutide treated participants but occurred with incidence  $\leq 10\%$ , not shown; 6.2% with Saxenda vs 0.8% with placebo discontinued treatment as a result of gastrointestinal adverse reactions; CNS: Dizziness, malaise and fatigue occurred were more also prevalent in liraglutide treated participants but occurred with incidence  $\leq 10\%$ , not shown; Metabolic: Liraglutide reduces blood glucose and thus, there is a potential for hypoglycaemia to occur. In the SCALE diabetes trial severe hypoglycemia occurred in 3 (0.7%) of 422 Saxenda-treated patients and in none of the 212 placebo-treated patients. In clinical trials involving patients without T2DM<sup>[42,43,205,206]</sup> no systematic reporting of hypoglycemia occurred but spontaneously reported symptomatic episodes potentially hypoglycemic in cause were reported by 1.6% (46/2962) of liraglutide 3 mg and 1.1% of (19/1729) placebo treated non diabetic patients. T2DM: Type 2 diabetes mellitus; GI: Gastrointestinal; CNS: Central nervous system.

co-morbidity in the United Kingdom.

## CONCLUSION

Obesity is a global epidemic, perhaps the greatest challenge to global and public health of our time. Whilst public health initiatives should continue to focus on curbing the projected upward trends in the incidence of obesity and overweight, effective management of those individuals already obese remains an important and as yet unmet clinical need. The current medical management of obesity in the United Kingdom is suboptimal, with the only treatment modality with proven long-term

efficacy being bariatric surgery. Both risky and costly, this treatment option is not viable for the widespread management of obesity, and remains reserved for extreme cases. The ideal medical management of any illness utilizes a targeted pharmacotherapy that either repletes physiological factors pathologically depleted, or antagonizes pathological processes, the development of such an agent requiring an understanding of the pathophysiology underpinning a disease. Though the pathophysiology of clinical obesity is undoubtedly multifaceted, several lines of clinical evidence implicate a role for functional impairments in GLP-1 signalling. Whilst genetic studies implicate a role for primary altered GLP-1



**Figure 17** Reported incidences of serious medical conditions from pooled analyses of 5 double-blinded placebo controlled trials studying the safety and efficacy of 3 mg liraglutide (Saxenda) in 3384 overweight or obese subjects receiving liraglutide and 1941 placebo controls. Hypotension associated adverse reactions (Hypo-ADR) refer to hypotension, orthostatic hypotension, circulatory collapse, and decreased blood pressure). One of the six (0.2%) liraglutide treated subjects reporting suicidal ideations attempted suicide. PTC: Papillary thyroid carcinoma; CRC: Colorectal carcinoma; Br Ca: Breast cancer; CCD: Cardiac conduction disorders.

signalling as a risk factor towards development of the obesity phenotype, clinical studies assessing physiological GLP-1 responses in normal weight and obese subjects suggest weight gain may induce functional deficits in GLP-1 that facilitates maintenance of the obesity phenotype. Whatever the relationship, cause or effect, reductions in functional GLP-1 signalling seems to play a role in clinical obesity, as such, the pharmacological replenishment of this functional deficit seems a promising target for the medical management of obesity in the clinic. Indeed, the GLP-1 analogue liraglutide 3 mg has shown promising results in achieving and maintaining greater weight loss in obese individuals when compared to control or currently licensed anti-obesity medication. Though results from extended phase III and phase IV studies report the development of potentially fatal adverse drug events in those randomized to or prescribed liraglutide 3 mg respectively, the scarcity of incidence and lack of causal relationship sees such potential risks overshadowed by the proven superior weight loss efficacy of treatment. Cost-benefit, however, may pose a barrier toward viable NHS funding, though this may be overcome by strategic treatment delivery; combining short-term liraglutide 3 mg treatment ( $\leq 36$  wk) with behavioural therapies targeted toward promoting healthy lifestyle changes. With drug induced weight loss potentially reinforcing adherence to long-term lifestyle changes, if successful, shortened in-treatment period alongside decreases in direct and indirect socioeconomic burdens of obesity and overweight secondary to achievement and maintenance of significant weight loss associates a long-term cost-benefit to funding treatment. Such a concept supports the use of liraglutide 3 mg as the first line anti-obesity agent on the NHS when conservative lifestyle management alone has failed in achieving clinically significant weight loss in comorbid overweight or obese adults.

## REFERENCES

- 1 **Organisation TWH.** Global health observatory data; Situation and trend, 2016. Available from: URL: [http://www.who.int/gho/ncd/risk\\_factors/obesity\\_text/en/](http://www.who.int/gho/ncd/risk_factors/obesity_text/en/)
- 2 **Organisation TWH.** Obesity and Overweight fact sheet. Jun 2016. Available from: URL: <http://www.who.int/mediacentre/factsheets/fs311/en/>
- 3 **Kelly T,** Yang W, Chen CS, Reynolds K, He J. Global burden of obesity in 2005 and projections to 2030. *Int J Obes (Lond)* 2008; **32**: 1431-1437 [PMID: 18607383 DOI: 10.1038/ijo.2008.102]
- 4 **Guh DP,** Zhang W, Bansback N, Amarsi Z, Birmingham CL, Anis AH. The incidence of co-morbidities related to obesity and overweight: a systematic review and meta-analysis. *BMC Public Health* 2009; **9**: 88 [PMID: 19320986 DOI: 10.1186/1471-2458-9-88]
- 5 **Puhl RM,** Heuer CA. Obesity stigma: important considerations for public health. *Am J Public Health* 2010; **100**: 1019-1028 [PMID: 20075322 DOI: 10.2105/AJPH.2009.159491]
- 6 **Kolotkin RL,** Meter K, Williams GR. Quality of life and obesity. *Obes Rev* 2001; **2**: 219-229 [PMID: 12119993 DOI: 10.1046/j.1467-789X.2001.00040.x]
- 7 **Luppino FS,** de Wit LM, Bouvy PF, Stijnen T, Cuijpers P, Penninx BW, Zitman FG. Overweight, obesity, and depression: a systematic review and meta-analysis of longitudinal studies. *Arch Gen Psychiatry* 2010; **67**: 220-229 [PMID: 20194822 DOI: 10.1001/archgenpsychiatry.2010.2]
- 8 **Vidal J.** Updated review on the benefits of weight loss. *Int J Obes Relat Metab Disord* 2002; **26** Suppl 4: S25-S28 [PMID: 12457296 DOI: 10.1038/sj.ijo.0802215]
- 9 **Goldstein DJ.** Beneficial health effects of modest weight loss. *Int J Obes Relat Metab Disord* 1992; **16**: 397-415 [PMID: 1322866]
- 10 **Blackburn G.** Effect of degree of weight loss on health benefits. *Obes Res* 1995; **3** Suppl 2: 211s-216s [PMID: 8581779]
- 11 **Grieve E,** Fenwick E, Yang HC, Lean M. The disproportionate economic burden associated with severe and complicated obesity: a systematic review. *Obes Rev* 2013; **14**: 883-894 [PMID: 23859626 DOI: 10.1111/obr.12059]
- 12 **Trogdon JG,** Finkelstein EA, Hylands T, Dellea PS, Kamal-Bahl SJ. Indirect costs of obesity: a review of the current literature. *Obes Rev* 2008; **9**: 489-500 [PMID: 18331420 DOI: 10.1111/j.1467-789X.2008.00472.x]
- 13 **Howard JT,** Potter LB. An assessment of the relationships between overweight, obesity, related chronic health conditions and worker absenteeism. *Obes Res Clin Pract* 2014; **8**: e1-15 [PMID: 24548572 DOI: 10.1016/j.orcp.2012.09.002]
- 14 **Ricci JA,** Chee E. Lost productive time associated with excess weight in the U.S. workforce. *J Occup Environ Med* 2005; **47**: 1227-1234 [PMID: 16340703 DOI: 10.1097/01.jom.0000184871.20901.c3]
- 15 **Specchia ML,** Veneziano MA, Caddeu C, Ferriero AM, Mancuso A, Iannuale C, Parente P, Capri S, Ricciardi W. Economic impact of adult obesity on health systems: a systematic review. *Eur J Public Health* 2015; **25**: 255-262 [PMID: 25320051 DOI: 10.1093/eurpub/cku170]
- 16 **Withrow D,** Alter DA. The economic burden of obesity world-



- wide: a systematic review of the direct costs of obesity. *Obes Rev* 2011; **12**: 131-141 [PMID: 20122135 DOI: 10.1111/j.1467-789X.2009.00712.x]
- 17 **Scarborough P**, Bhatnagar P, Wickramasinghe KK, Allender S, Foster C, Rayner M. The economic burden of ill health due to diet, physical inactivity, smoking, alcohol and obesity in the UK: an update to 2006-07 NHS costs. *J Public Health (Oxf)* 2011; **33**: 527-535 [PMID: 21562029 DOI: 10.1093/pubmed/fdr033]
- 18 **McPherson K**, Marsh TN. Foresight Tackling Obesity: Future Choices - Modelling Future Trends in Obesity and the Impact on Health (GOV UK). 2007
- 19 **Collins B**, Capewell S, O'Flaherty M, Timpson H, Razzaq A, Cheater S, Ireland R, Bromley H. Modelling the Health Impact of an English Sugary Drinks Duty at National and Local Levels. *PLoS One* 2015; **10**: e0130770 [PMID: 26121677 DOI: 10.1371/journal.pone.0130770]
- 20 **National Institute for Clinical Excellence T**. Obesity: Identification, assessment and management of overweight and obesity in children, young people and adults. 2014
- 21 **Dombrowski SU**, Knittle K, Avenell A, Araújo-Soares V, Sniehotta FF. Long term maintenance of weight loss with non-surgical interventions in obese adults: systematic review and meta-analyses of randomised controlled trials. *BMJ* 2014; **348**: g2646 [PMID: 25134100 DOI: 10.1136/bmj.g2646]
- 22 **Holzapfel C**, Hauner H. [Weight maintenance after weight loss - how the body defends its weight]. *Dtsch Med Wochenschr* 2011; **136**: 89-94 [PMID: 21225556 DOI: 10.1055/s-0030-1269445]
- 23 **Loveman E**, Frampton GK, Shepherd J, Picot J, Cooper K, Bryant J, Welch K, Clegg A. The clinical effectiveness and cost-effectiveness of long-term weight management schemes for adults: a systematic review. *Health Technol Assess* 2011; **15**: 1-182 [PMID: 21247515 DOI: 10.3310/hta15020]
- 24 **Greenway FL**. Physiological adaptations to weight loss and factors favouring weight regain. *Int J Obes (Lond)* 2015; **39**: 1188-1196 [PMID: 25896063 DOI: 10.1038/ijo.2015.59]
- 25 **Derosa G**, Maffioli P. Anti-obesity drugs: a review about their effects and their safety. *Expert Opin Drug Saf* 2012; **11**: 459-471 [PMID: 22439841 DOI: 10.1517/14740338.2012.675326]
- 26 **Kang JG**, Park CY. Anti-Obesity Drugs: A Review about Their Effects and Safety. *Diabetes Metab J* 2012; **36**: 13-25 [PMID: 22363917 DOI: 10.4093/dmj.2012.36.1.13]
- 27 **Yanovski SZ**, Yanovski JA. Obesity. *N Engl J Med* 2002; **346**: 591-602 [PMID: 11856799 DOI: 10.1056/NEJMr012586]
- 28 **Golomb I**, Ben David M, Glass A, Kolitz T, Keidar A. Long-term Metabolic Effects of Laparoscopic Sleeve Gastrectomy. *JAMA Surg* 2015; **150**: 1051-1057 [PMID: 26244446 DOI: 10.1001/jamasurg.2015.2202]
- 29 **Hirth DA**, Jones EL, Rothchild KB, Mitchell BC, Schoen JA. Laparoscopic sleeve gastrectomy: long-term weight loss outcomes. *Surg Obes Relat Dis* 2015; **11**: 1004-1007 [PMID: 25980329 DOI: 10.1016/j.soard.2015.02.016]
- 30 **Costa RC**, Yamaguchi N, Santo MA, Riccioppo D, Pinto-Junior PE. Outcomes on quality of life, weight loss, and comorbidities after Roux-en-Y gastric bypass. *Arq Gastroenterol* 2014; **51**: 165-170 [PMID: 25296074 DOI: 10.1590/S0004-28032014000300002]
- 31 **Pasulka PS**, Bistrian BR, Benotti PN, Blackburn GL. The risks of surgery in obese patients. *Ann Intern Med* 1986; **104**: 540-546 [PMID: 3513685 DOI: 10.7326/0003-4819-104-4-540]
- 32 **Dority J**, Hassan ZU, Chau D. Anesthetic implications of obesity in the surgical patient. *Clin Colon Rectal Surg* 2011; **24**: 222-228 [PMID: 23204937 DOI: 10.1055/s-0031-1295685]
- 33 **Nishida C**, Uauy R, Kumanyika S, Shetty P. The joint WHO/FAO expert consultation on diet, nutrition and the prevention of chronic diseases: process, product and policy implications. *Public Health Nutr* 2004; **7**: 245-250 [PMID: 14972063 DOI: 10.1079/PHN2003592]
- 34 **Woods SC**, D'Alessio DA. Central control of body weight and appetite. *J Clin Endocrinol Metab* 2008; **93**: S37-S50 [PMID: 18987269 DOI: 10.1210/jc.2008-1630]
- 35 **Berthoud HR**. Metabolic and hedonic drives in the neural control of appetite: who is the boss? *Curr Opin Neurobiol* 2011; **21**: 888-896 [PMID: 21981809 DOI: 10.1016/j.conb.2011.09.004]
- 36 **Williams DL**. Neural integration of satiation and food reward: role of GLP-1 and orexin pathways. *Physiol Behav* 2014; **136**: 194-199 [PMID: 24650552 DOI: 10.1016/j.physbeh.2014.03.013]
- 37 **Skibicka KP**. The central GLP-1: implications for food and drug reward. *Front Neurosci* 2013; **7**: 181 [PMID: 24133407 DOI: 10.3389/fnins.2013.00181]
- 38 **Prasad-Reddy L**, Isaacs D. A clinical review of GLP-1 receptor agonists: efficacy and safety in diabetes and beyond. *Drugs Context* 2015; **4**: 212283 [PMID: 26213556 DOI: 10.7573/dic.212283]
- 39 **Monami M**, Dicembrini I, Marchionni N, Rotella CM, Mannucci E. Effects of glucagon-like peptide-1 receptor agonists on body weight: a meta-analysis. *Exp Diabetes Res* 2012; **2012**: 672658 [PMID: 22675341 DOI: 10.1155/2012/672658]
- 40 **Vilsbøll T**, Christensen M, Junker AE, Knop FK, Gluud LL. Effects of glucagon-like peptide-1 receptor agonists on weight loss: systematic review and meta-analyses of randomised controlled trials. *BMJ* 2012; **344**: d7771 [PMID: 22236411 DOI: 10.1136/bmj.d7771]
- 41 **Collier A**, Blackman A, Foster G, Zammit G, Rosenberg R, Wadden T, Aronne L, Claudius B, Jensen T, Mignot E. S28 Liraglutide 3.0 Mg Reduces Severity Of Obstructive Sleep Apnoea And Body Weight In Obese Individuals With Moderate Or Severe Disease: Scale Sleep Apnoea Trial. *Thorax* 2014; **69** Suppl 2: A16-A17 [DOI: 10.1016/j.jcjd.2015.01.139]
- 42 **Astrup A**, Carraro R, Finer N, Harper A, Kunesova M, Lean ME, Niskanen L, Rasmussen MF, Rissanen A, Rössner S, Savolainen MJ, Van Gaal L. Safety, tolerability and sustained weight loss over 2 years with the once-daily human GLP-1 analog, liraglutide. *Int J Obes (Lond)* 2012; **36**: 843-854 [PMID: 21844879 DOI: 10.1038/ijo.2011.158]
- 43 **Astrup A**, Rössner S, Van Gaal L, Rissanen A, Niskanen L, Al Hakim M, Madsen J, Rasmussen MF, Lean ME. Effects of liraglutide in the treatment of obesity: a randomised, double-blind, placebo-controlled study. *Lancet* 2009; **374**: 1606-1616 [PMID: 19853906 DOI: 10.1016/S0140-6736(09)61375-1]
- 44 **Liraglutide 3.0 mg for Weight Management**; briefing document. 2014
- 45 **Novo Nordisk**. Saxenda® approved in Europe for the treatment of obesity. 2015, March 23. Available from: URL: <http://www.novonordisk.com/bin/getPDF.1905678.pdf>
- 46 **UK Medicines Information**. New drugs online: Liraglutide. 2015
- 47 **Gao Q**, Horvath TL. Neurobiology of feeding and energy expenditure. *Annu Rev Neurosci* 2007; **30**: 367-398 [PMID: 17506645 DOI: 10.1146/annurev.neuro.30.051606.094324]
- 48 **Zhang Y**, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994; **372**: 425-432 [PMID: 7984236 DOI: 10.1038/372425a0]
- 49 **Volkow ND**, Wang GJ, Baler RD. Reward, dopamine and the control of food intake: implications for obesity. *Trends Cogn Sci* 2011; **15**: 37-46 [PMID: 21109477 DOI: 10.1016/j.tics.2010.11.001]
- 50 **Secher A**, Jelsing J, Baquero AF, Hecksher-Sørensen J, Cowley MA, Dalbøge LS, Hansen G, Grove KL, Pyke C, Raun K, Schäffer L, Tang-Christensen M, Verma S, Witgen BM, Vrang N, Bjerre Knudsen L. The arcuate nucleus mediates GLP-1 receptor agonist liraglutide-dependent weight loss. *J Clin Invest* 2014; **124**: 4473-4488 [PMID: 25202980 DOI: 10.1172/JCI75276]
- 51 **Katsurada K**, Maejima Y, Nakata M, Kodaira M, Suyama S, Iwasaki Y, Kario K, Yada T. Endogenous GLP-1 acts on paraventricular nucleus to suppress feeding: projection from nucleus tractus solitarius and activation of corticotropin-releasing hormone, nesfatin-1 and oxytocin neurons. *Biochem Biophys Res Commun* 2014; **451**: 276-281 [PMID: 25089000 DOI: 10.1016/j.bbrc.2014.07.116]
- 52 **Koob GF**, Volkow ND. Neurocircuitry of addiction. *Neuropsychopharmacology* 2010; **35**: 217-238 [PMID: 19710631 DOI: 10.1038/npp.2009.110]
- 53 **Figlewicz DP**. Adiposity signals and food reward: expanding the

- CNS roles of insulin and leptin. *Am J Physiol Regul Integr Comp Physiol* 2003; **284**: R882-892 [PMID: 12626355 DOI: 10.1152/ajpregu.00602.2002]
- 54 **Ulrich-Lai YM**, Ryan KK. Neuroendocrine circuits governing energy balance and stress regulation: functional overlap and therapeutic implications. *Cell Metab* 2014; **19**: 910-925 [PMID: 24630812 DOI: 10.1016/j.cmet.2014.01.020]
- 55 **Broberger C**, Johansen J, Johansson C, Schalling M, Hökfelt T. The neuropeptide Y/agouti gene-related protein (AGRP) brain circuitry in normal, anorectic, and monosodium glutamate-treated mice. *Proc Natl Acad Sci USA* 1998; **95**: 15043-15048 [PMID: 9844012]
- 56 **Hahn TM**, Breininger JF, Baskin DG, Schwartz MW. Coexpression of Agrp and NPY in fasting-activated hypothalamic neurons. *Nat Neurosci* 1998; **1**: 271-272 [PMID: 10195157 DOI: 10.1038/1082]
- 57 **Millington GW**. The role of proopiomelanocortin (POMC) neurones in feeding behaviour. *Nutr Metab (Lond)* 2007; **4**: 18 [PMID: 17764572 DOI: 10.1186/1743-7075-4-18]
- 58 **Dhillon WS**, Small CJ, Stanley SA, Jethwa PH, Seal LJ, Murphy KG, Ghatei MA, Bloom SR. Hypothalamic interactions between neuropeptide Y, agouti-related protein, cocaine- and amphetamine-regulated transcript and alpha-melanocyte-stimulating hormone in vitro in male rats. *J Neuroendocrinol* 2002; **14**: 725-730 [PMID: 12213133]
- 59 **Haskell-Luevano C**, Hendrata S, North C, Sawyer TK, Hadley ME, Hrubby VJ, Dickinson C, Gantz I. Discovery of prototype peptidomimetic agonists at the human melanocortin receptors MC1R and MC4R. *J Med Chem* 1997; **40**: 2133-2139 [PMID: 9216831 DOI: 10.1021/jm960840h]
- 60 **Nijenhuis WA**, Oosterom J, Adan RA. AgRP(83-132) acts as an inverse agonist on the human-melanocortin-4 receptor. *Mol Endocrinol* 2001; **15**: 164-171 [PMID: 11145747 DOI: 10.1210/mend.15.1.0578]
- 61 **Haskell-Luevano C**, Monck EK. Agouti-related protein functions as an inverse agonist at a constitutively active brain melanocortin-4 receptor. *Regul Pept* 2001; **99**: 1-7 [PMID: 11257308]
- 62 **Broberger C**, Hökfelt T. Hypothalamic and vagal neuropeptide circuitries regulating food intake. *Physiol Behav* 2001; **74**: 669-682 [PMID: 11790430 DOI: 10.1016/S0031-9384(01)00611-4]
- 63 **Cowley MA**, Pronchuk N, Fan W, Dinulescu DM, Colmers WF, Cone RD. Integration of NPY, AGRP, and melanocortin signals in the hypothalamic paraventricular nucleus: evidence of a cellular basis for the adipostat. *Neuron* 1999; **24**: 155-163 [PMID: 10677034 DOI: 10.1016/S0896-6273(00)80829-6]
- 64 **Richard JE**, Anderberg RH, Göteson A, Gribble FM, Reimann F, Skibicka KP. Activation of the GLP-1 receptors in the nucleus of the solitary tract reduces food reward behavior and targets the mesolimbic system. *PLoS One* 2015; **10**: e0119034 [PMID: 25793511 DOI: 10.1371/journal.pone.0119034]
- 65 **Kennedy GC**. The role of depot fat in the hypothalamic control of food intake in the rat. *Proc R Soc Lond B Biol Sci* 1953; **140**: 578-596 [PMID: 13027283]
- 66 **Drucker DJ**, Asa S. Glucagon gene expression in vertebrate brain. *J Biol Chem* 1988; **263**: 13475-13478 [PMID: 2901414]
- 67 **Lee YC**, Brubaker PL, Drucker DJ. Developmental and tissue-specific regulation of proglucagon gene expression. *Endocrinology* 1990; **127**: 2217-2222 [PMID: 2226310 DOI: 10.1210/endo-127-5-2217]
- 68 **Merchenthaler I**, Lane M, Shughrue P. Distribution of pre-pro-glucagon and glucagon-like peptide-1 receptor messenger RNAs in the rat central nervous system. *J Comp Neurol* 1999; **403**: 261-280 [PMID: 9886047]
- 69 **Mojsov S**, Heinrich G, Wilson IB, Ravazzola M, Orci L, Habener JF. Preproglucagon gene expression in pancreas and intestine diversifies at the level of post-translational processing. *J Biol Chem* 1986; **261**: 11880-11889 [PMID: 3528148]
- 70 **Baggio LL**, Drucker DJ. Biology of incretins: GLP-1 and GIP. *Gastroenterology* 2007; **132**: 2131-2157 [PMID: 17498508 DOI: 10.1053/j.gastro.2007.03.054]
- 71 **Holst JJ**. The physiology of glucagon-like peptide 1. *Physiol Rev* 2007; **87**: 1409-1439 [PMID: 17928588 DOI: 10.1152/physrev.00034.2006]
- 72 **Pocai A**. Unraveling oxyntomodulin, GLP1's enigmatic brother. *J Endocrinol* 2012; **215**: 335-346 [PMID: 23019069 DOI: 10.1530/JOE-12-0368]
- 73 **Orskov C**, Holst JJ, Knuhtsen S, Baldissera FG, Poulsen SS, Nielsen OV. Glucagon-like peptides GLP-1 and GLP-2, predicted products of the glucagon gene, are secreted separately from pig small intestine but not pancreas. *Endocrinology* 1986; **119**: 1467-1475 [PMID: 3530719 DOI: 10.1210/endo-119-4-1467]
- 74 **Rocca AS**, Brubaker PL. Role of the vagus nerve in mediating proximal nutrient-induced glucagon-like peptide-1 secretion. *Endocrinology* 1999; **140**: 1687-1694 [PMID: 10098504 DOI: 10.1210/endo.140.4.6643]
- 75 **Tucker JD**, Dhanvantari S, Brubaker PL. Proglucagon processing in islet and intestinal cell lines. *Regul Pept* 1996; **62**: 29-35 [PMID: 8738879 DOI: 10.1016/0167-0115(95)00167-0]
- 76 **Bryant MG**, Bloom SR, Polak JM, Hobbs S, Domschke W, Domschke S, Mitznegg P, Ruppini H, Demling L. Measurement of gut hormonal peptides in biopsies from human stomach and proximal small intestine. *Gut* 1983; **24**: 114-119 [PMID: 6343197]
- 77 **Eissele R**, Göke R, Willemer S, Harthus HP, Vermeer H, Arnold R, Göke B. Glucagon-like peptide-1 cells in the gastrointestinal tract and pancreas of rat, pig and man. *Eur J Clin Invest* 1992; **22**: 283-291 [PMID: 1499644 DOI: 10.1111/j.1365-2362.1992.tb01464.x]
- 78 **Knudsen JB**, Holst JJ, Asnaes S, Johansen A. Identification of cells with pancreatic-type and gut-type glucagon immunoreactivity in the human colon. *Acta Pathol Microbiol Scand A* 1975; **83**: 741-743 [PMID: 1189925 DOI: 10.1111/j.1699-0463.1975.tb01407.x]
- 79 **O'Donovan DG**, Doran S, Feinle-Bisset C, Jones KL, Meyer JH, Wishart JM, Morris HA, Horowitz M. Effect of variations in small intestinal glucose delivery on plasma glucose, insulin, and incretin hormones in healthy subjects and type 2 diabetes. *J Clin Endocrinol Metab* 2004; **89**: 3431-3435 [PMID: 15240627 DOI: 10.1210/jc.2004-0334]
- 80 **Anini Y**, Hansotia T, Brubaker PL. Muscarinic receptors control postprandial release of glucagon-like peptide-1: in vivo and in vitro studies in rats. *Endocrinology* 2002; **143**: 2420-2426 [PMID: 12021207 DOI: 10.1210/endo.143.6.8840]
- 81 **Hansen L**, Deacon CF, Orskov C, Holst JJ. Glucagon-like peptide-1-(7-36)amide is transformed to glucagon-like peptide-1-(9-36)amide by dipeptidyl peptidase IV in the capillaries supplying the L cells of the porcine intestine. *Endocrinology* 1999; **140**: 5356-5363 [PMID: 10537167 DOI: 10.1210/endo.140.11.7143]
- 82 **Herrmann C**, Göke R, Richter G, Fehmann HC, Arnold R, Göke B. Glucagon-like peptide-1 and glucose-dependent insulin-releasing polypeptide plasma levels in response to nutrients. *Digestion* 1995; **56**: 117-126 [PMID: 7750665]
- 83 **Pais R**, Gribble FM, Reimann F. Signalling pathways involved in the detection of peptones by murine small intestinal enteroendocrine L-cells. *Peptides* 2016; **77**: 9-15 [PMID: 26215048 DOI: 10.1016/j.peptides.2015.07.019]
- 84 **Theodorakis MJ**, Carlson O, Michopoulos S, Doyle ME, Juhaszova M, Petraki K, Egan JM. Human duodenal enteroendocrine cells: source of both incretin peptides, GLP-1 and GIP. *Am J Physiol Endocrinol Metab* 2006; **290**: E550-E559 [PMID: 16219666 DOI: 10.1152/ajpendo.00326.2004]
- 85 **Habib AM**, Richards P, Cairns LS, Rogers GJ, Bannon CA, Parker HE, Morley TC, Yeo GS, Reimann F, Gribble FM. Overlap of endocrine hormone expression in the mouse intestine revealed by transcriptional profiling and flow cytometry. *Endocrinology* 2012; **153**: 3054-3065 [PMID: 22685263 DOI: 10.1210/en.2011-2170]
- 86 **Cho YM**, Fujita Y, Kieffer TJ. Glucagon-like peptide-1: glucose homeostasis and beyond. *Annu Rev Physiol* 2014; **76**: 535-559 [PMID: 24245943 DOI: 10.1146/annurev-physiol-021113-170315]
- 87 **Lim GE**, Brubaker PL. Glucagon-like peptide 1 secretion by the L-cell. *Diabetes* 2006; **55** Suppl 2: S70
- 88 **Kieffer TJ**, McIntosh CH, Pederson RA. Degradation of glucose-

- dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 in vitro and in vivo by dipeptidyl peptidase IV. *Endocrinology* 1995; **136**: 3585-3596 [PMID: 7628397 DOI: 10.1210/endo.136.8.7628397]
- 89 Kieffer TJ, Habener JF. The glucagon-like peptides. *Endocr Rev* 1999; **20**: 876-913 [PMID: 10605628 DOI: 10.1210/edrv.20.6.0385]
- 90 Deacon CF, Pridal L, Klarskov L, Olesen M, Holst JJ. Glucagon-like peptide 1 undergoes differential tissue-specific metabolism in the anesthetized pig. *Am J Physiol* 1996; **271**: E458-E464 [PMID: 8843738]
- 91 Donnelly D. The structure and function of the glucagon-like peptide-1 receptor and its ligands. *Br J Pharmacol* 2012; **166**: 27-41 [PMID: 21950636 DOI: 10.1111/j.1476-5381.2011.01687.x]
- 92 Baggio LL, Drucker DJ. Biology of incretins: GLP-1 and GIP. *Gastroenterology* 2007; **132**: 2131-2157 [PMID: 17498508 DOI: 10.1053/j.gastro.2007.03.054]
- 93 Cho YM, Fujita Y, Kieffer TJ. Glucagon-like peptide-1: glucose homeostasis and beyond. *Annu Rev Physiol* 2014; **76**: 535-559 [PMID: 24245943 DOI: 10.1146/annurev-physiol-021113-170315]
- 94 Näslund E, Gutniak M, Skogar S, Rössner S, Hellström PM. Glucagon-like peptide 1 increases the period of postprandial satiety and slows gastric emptying in obese men. *Am J Clin Nutr* 1998; **68**: 525-530 [PMID: 9734726]
- 95 Flint A, Raben A, Astrup A, Holst JJ. Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. *J Clin Invest* 1998; **101**: 515-520 [PMID: 9449682 DOI: 10.1172/JCI990]
- 96 Cegla J, Troke RC, Jones B, Tharakan G, Kenkre J, McCullough KA, Lim CT, Parvizi N, Hussein M, Chambers ES, Minnion J, Cuenco J, Ghatei MA, Meeran K, Tan TM, Bloom SR. Coinfusion of low-dose GLP-1 and glucagon in man results in a reduction in food intake. *Diabetes* 2014; **63**: 3711-3720 [PMID: 24939425 DOI: 10.2337/db14-0242]
- 97 Flint A, Raben A, Astrup A, Holst JJ. Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. *J Clin Invest* 1998; **101**: 515-520 [PMID: 9449682 DOI: 10.1172/JCI990]
- 98 Long SJ, Sutton JA, Amae WB, Giouvanoudi A, Spyrou NM, Rogers PJ, Morgan LM. No effect of glucagon-like peptide-1 on short-term satiety and energy intake in man. *Br J Nutr* 1999; **81**: 273-279 [PMID: 10999014]
- 99 Gutzwiller JP, Drewe J, Göke B, Schmidt H, Rohrer B, Lareida J, Beglinger C. Glucagon-like peptide-1 promotes satiety and reduces food intake in patients with diabetes mellitus type 2. *Am J Physiol* 1999; **276**: R1541-R1544 [PMID: 10233049]
- 100 Verdich C, Flint A, Gutzwiller JP, Näslund E, Beglinger C, Hellström PM, Long SJ, Morgan LM, Holst JJ, Astrup A. A meta-analysis of the effect of glucagon-like peptide-1 (7-36) amide on ad libitum energy intake in humans. *J Clin Endocrinol Metab* 2001; **86**: 4382-4389 [PMID: 11549680 DOI: 10.1210/jcem.86.9.7877]
- 101 Pannacchiulli N, Bunt JC, Koska J, Bogardus C, Krakoff J. Higher fasting plasma concentrations of glucagon-like peptide 1 are associated with higher resting energy expenditure and fat oxidation rates in humans. *Am J Clin Nutr* 2006; **84**: 556-560 [PMID: 16960169]
- 102 Flint A, Raben A, Rehfeld JF, Holst JJ, Astrup A. The effect of glucagon-like peptide-1 on energy expenditure and substrate metabolism in humans. *Int J Obes Relat Metab Disord* 2000; **24**: 288-298 [PMID: 10757621]
- 103 Shalev A, Holst JJ, Keller U. Effects of glucagon-like peptide 1 (7-36 amide) on whole-body protein metabolism in healthy man. *Eur J Clin Invest* 1997; **27**: 10-16 [PMID: 9041371]
- 104 Näslund E, Barkeling B, King N, Gutniak M, Blundell JE, Holst JJ, Rössner S, Hellström PM. Energy intake and appetite are suppressed by glucagon-like peptide-1 (GLP-1) in obese men. *Int J Obes Relat Metab Disord* 1999; **23**: 304-311 [PMID: 10193877 DOI: 10.1038/sj.ijo.0800818]
- 105 Broide E, Bloch O, Ben-Yehudah G, Cantrell D, Shirin H, Rapoport MJ. GLP-1 receptor is expressed in human stomach mucosa: analysis of its cellular association and distribution within gastric glands. *J Histochem Cytochem* 2013; **61**: 649-658 [PMID: 23803499 DOI: 10.1369/0022155413497586]
- 106 Tornehave D, Kristensen P, Rømer J, Knudsen LB, Heller RS. Expression of the GLP-1 receptor in mouse, rat, and human pancreas. *J Histochem Cytochem* 2008; **56**: 841-851 [PMID: 18541709 DOI: 10.1369/jhc.2008.951319]
- 107 Travagli RA, Hermann GE, Browning KN, Rogers RC. Brainstem circuits regulating gastric function. *Annu Rev Physiol* 2006; **68**: 279-305 [PMID: 16460274 DOI: 10.1146/annurev.physiol.68.040504.094635]
- 108 Hunt JN. A possible relation between the regulation of gastric emptying and food intake. *Am J Physiol* 1980; **239**: G1-G4 [PMID: 7395999]
- 109 Di Lorenzo C, Williams CM, Hajnal F, Valenzuela JE. Pectin delays gastric emptying and increases satiety in obese subjects. *Gastroenterology* 1988; **95**: 1211-1215 [PMID: 3169489 DOI: 10.1016/0016-5085(88)90352-6]
- 110 Clegg ME, Ranawana V, Shafat A, Henry CJ. Soups increase satiety through delayed gastric emptying yet increased glycaemic response. *Eur J Clin Nutr* 2013; **67**: 8-11 [PMID: 23093339 DOI: 10.1038/ejcn.2012.152]
- 111 Hlebowicz J, Darwiche G, Björgell O, Almér LO. Effect of cinnamon on postprandial blood glucose, gastric emptying, and satiety in healthy subjects. *Am J Clin Nutr* 2007; **85**: 1552-1556 [PMID: 17556692]
- 112 Hlebowicz J, Hlebowicz A, Lindstedt S, Björgell O, Höglund P, Holst JJ, Darwiche G, Almér LO. Effects of 1 and 3 g cinnamon on gastric emptying, satiety, and postprandial blood glucose, insulin, glucose-dependent insulinotropic polypeptide, glucagon-like peptide 1, and ghrelin concentrations in healthy subjects. *Am J Clin Nutr* 2009; **89**: 815-821 [PMID: 19158209 DOI: 10.3945/ajcn.2008.26807]
- 113 Nauck MA, Niedereichholz U, Ettler R, Holst JJ, Orskov C, Ritzel R, Schmiegell WH. Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. *Am J Physiol* 1997; **273**: E981-E988 [PMID: 9374685]
- 114 Schirra J, Wank U, Arnold R, Göke B, Katschinski M. Effects of glucagon-like peptide-1(7-36)amide on motility and sensation of the proximal stomach in humans. *Gut* 2002; **50**: 341-348 [PMID: 11839712 DOI: 10.1136/gut.50.3.341]
- 115 Little TJ, Pilchiewicz AN, Russo A, Phillips L, Jones KL, Nauck MA, Wishart J, Horowitz M, Feinle-Bisset C. Effects of intravenous glucagon-like peptide-1 on gastric emptying and intragastric distribution in healthy subjects: relationships with postprandial glycemic and insulinemic responses. *J Clin Endocrinol Metab* 2006; **91**: 1916-1923 [PMID: 16492694 DOI: 10.1210/jc.2005-2220]
- 116 Meier JJ, Gallwitz B, Salmen S, Goetze O, Holst JJ, Schmidt WE, Nauck MA. Normalization of glucose concentrations and deceleration of gastric emptying after solid meals during intravenous glucagon-like peptide 1 in patients with type 2 diabetes. *J Clin Endocrinol Metab* 2003; **88**: 2719-2725 [PMID: 12788879 DOI: 10.1210/jc.2003-030049]
- 117 Vrang N, Phifer CB, Corkern MM, Berthoud HR. Gastric distension induces c-Fos in medullary GLP-1/2-containing neurons. *Am J Physiol Regul Integr Comp Physiol* 2003; **285**: R470-R478 [PMID: 12714357 DOI: 10.1152/ajpregu.00732.2002]
- 118 Polonsky KS, Given BD, Hirsch L, Shapiro ET, Tillil H, Beebe C, Galloway JA, Frank BH, Karrison T, Van Cauter E. Quantitative study of insulin secretion and clearance in normal and obese subjects. *J Clin Invest* 1988; **81**: 435-441 [PMID: 3276729 DOI: 10.1172/JCI113338]
- 119 Benedict C, Kern W, Schultes B, Born J, Hallschmid M. Differential sensitivity of men and women to anorexigenic and memory-improving effects of intranasal insulin. *J Clin Endocrinol Metab* 2008; **93**: 1339-1344 [PMID: 18230654 DOI: 10.1210/jc.2007-2606]
- 120 Niswender KD, Morrison CD, Clegg DJ, Olson R, Baskin DG, Myers MG, Seeley RJ, Schwartz MW. Insulin activation of phosphatidylinositol 3-kinase in the hypothalamic arcuate nucleus: a key mediator of insulin-induced anorexia. *Diabetes* 2003; **52**: 227-231 [PMID: 12540590 DOI: 10.2337/diabetes.52.2.227]



- 121 **Watanabe M**, Hayasaki H, Tamayama T, Shimada M. Histologic distribution of insulin and glucagon receptors. *Braz J Med Biol Res* 1998; **31**: 243-256 [PMID: 9686147]
- 122 **Iñiguez SD**, Warren BL, Neve RL, Nestler EJ, Russo SJ, Bolaños-Guzmán CA. Insulin receptor substrate-2 in the ventral tegmental area regulates behavioral responses to cocaine. *Behav Neurosci* 2008; **122**: 1172-1177 [PMID: 18823173 DOI: 10.1037/a0012893]
- 123 **Meloni AR**, DeYoung MB, Lowe C, Parkes DG. GLP-1 receptor activated insulin secretion from pancreatic  $\beta$ -cells: mechanism and glucose dependence. *Diabetes Obes Metab* 2013; **15**: 15-27 [PMID: 22776039 DOI: 10.1111/j.1463-1326.2012.01663.x]
- 124 **Wang Q**, Liu C, Uchida A, Chuang JC, Walker A, Liu T, Osborne-Lawrence S, Mason BL, Mosher C, Berglund ED, Elmquist JK, Zigman JM. Arcuate AgRP neurons mediate orexigenic and glucoregulatory actions of ghrelin. *Mol Metab* 2014; **3**: 64-72 [PMID: 24567905 DOI: 10.1016/j.molmet.2013.10.001]
- 125 **Skibicka KP**, Hansson C, Alvarez-Crespo M, Friberg PA, Dickson SL. Ghrelin directly targets the ventral tegmental area to increase food motivation. *Neuroscience* 2011; **180**: 129-137 [PMID: 21335062 DOI: 10.1016/j.neuroscience.2011.02.016]
- 126 **Hagemann D**, Holst JJ, Gethmann A, Banasch M, Schmidt WE, Meier JJ. Glucagon-like peptide 1 (GLP-1) suppresses ghrelin levels in humans via increased insulin secretion. *Regul Pept* 2007; **143**: 64-68 [PMID: 17434608 DOI: 10.1016/j.regpep.2007.03.002]
- 127 **Saad MF**, Bernaba B, Hwu CM, Jinagouda S, Fahmi S, Kogosov E, Boyadjian R. Insulin regulates plasma ghrelin concentration. *J Clin Endocrinol Metab* 2002; **87**: 3997-4000 [PMID: 12161550 DOI: 10.1210/jcem.87.8.8879]
- 128 **Wei Y**, Mojsov S. Tissue-specific expression of the human receptor for glucagon-like peptide-I: brain, heart and pancreatic forms have the same deduced amino acid sequences. *FEBS Lett* 1995; **358**: 219-224 [PMID: 7843404]
- 129 **Alvarez E**, Martínez MD, Roncero I, Chowen JA, García-Cuartero B, Gispert JD, Sanz C, Vázquez P, Maldonado A, de Cáceres J, Desco M, Pozo MA, Blázquez E. The expression of GLP-1 receptor mRNA and protein allows the effect of GLP-1 on glucose metabolism in the human hypothalamus and brainstem. *J Neurochem* 2005; **92**: 798-806 [PMID: 15686481 DOI: 10.1111/j.1471-4159.2004.02914.x]
- 130 **Pannacciulli N**, Le DS, Salbe AD, Chen K, Reiman EM, Tataranni PA, Krakoff J. Postprandial glucagon-like peptide-1 (GLP-1) response is positively associated with changes in neuronal activity of brain areas implicated in satiety and food intake regulation in humans. *Neuroimage* 2007; **35**: 511-517 [PMID: 17317222 DOI: 10.1016/j.neuroimage.2006.12.035]
- 131 **De Silva A**, Salem V, Long CJ, Makwana A, Newbould RD, Rabiner EA, Ghatei MA, Bloom SR, Matthews PM, Beaver JD, Dhillon WS. The gut hormones PYY 3-36 and GLP-1 7-36 amide reduce food intake and modulate brain activity in appetite centers in humans. *Cell Metab* 2011; **14**: 700-706 [PMID: 22000927 DOI: 10.1016/j.cmet.2011.09.010]
- 132 **Astrup A**. Thermogenesis in human brown adipose tissue and skeletal muscle induced by sympathomimetic stimulation. *Acta Endocrinol Suppl (Copenh)* 1986; **278**: 1-32 [PMID: 3464154]
- 133 **Astrup A**, Bülow J, Madsen J, Christensen NJ. Contribution of BAT and skeletal muscle to thermogenesis induced by ephedrine in man. *Am J Physiol* 1985; **248**: E507-E515 [PMID: 3922230]
- 134 **Welle S**. Sympathetic nervous system response to intake. *Am J Clin Nutr* 1995; **62**: 1118S-1122S [PMID: 7484930]
- 135 **Spraul M**, Ravussin E, Fontvieille AM, Rising R, Larson DE, Anderson EA. Reduced sympathetic nervous activity. A potential mechanism predisposing to body weight gain. *J Clin Invest* 1993; **92**: 1730-1735 [PMID: 8408625 DOI: 10.1172/JCI116760]
- 136 **Bharucha AE**, Charkoudian N, Andrews CN, Camilleri M, Sletten D, Zinsmeister AR, Low PA. Effects of glucagon-like peptide-1, yohimbine, and nitrergic modulation on sympathetic and parasympathetic activity in humans. *Am J Physiol Regul Integr Comp Physiol* 2008; **295**: R874-R880 [PMID: 18596108 DOI: 10.1152/ajpregu.00153.2008]
- 137 **Huvenne H**, Dubern B. Molecular Mechanisms Underpinning the Development of Obesity; Monogenic Forms of Obesity. Switzerland: Springer International Publishing, 2014 [DOI: 10.1007/978-3-319-12766-8]
- 138 **Chung WK**. An overview of monogenic and syndromic obesities in humans. *Pediatr Blood Cancer* 2012; **58**: 122-128 [PMID: 21994130 DOI: 10.1002/pbc.23372]
- 139 **Doche ME**, Bochukova EG, Su HW, Pearce LR, Keogh JM, Henning E, Cline JM, Saeed S, Dale A, Cheetham T, Barroso I, Argetsinger LS, O'Rahilly S, Rui L, Carter-Su C, Farooqi IS. Human SH2B1 mutations are associated with maladaptive behaviors and obesity. *J Clin Invest* 2012; **122**: 4732-4736 [PMID: 23160192 DOI: 10.1172/JCI62696]
- 140 **Mencarelli M**, Dubern B, Alili R, Maestrini S, Benajiba L, Tagliaferri M, Galan P, Rinaldi M, Simon C, Tounian P, Herberg S, Liuzzi A, Di Blasio AM, Clement K. Rare melanocortin-3 receptor mutations with in vitro functional consequences are associated with human obesity. *Hum Mol Genet* 2011; **20**: 392-399 [PMID: 21047972 DOI: 10.1093/hmg/ddq472]
- 141 **Yazdi FT**, Clee SM, Meyre D. Obesity genetics in mouse and human: back and forth, and back again. *PeerJ* 2015; **3**: e856 [PMID: 25825681 DOI: 10.7717/peerj.856]
- 142 **Farooqi IS**. Monogenic human obesity. *Front Horm Res* 2008; **36**: 1-11 [PMID: 18230891 DOI: 10.1159/0000115333]
- 143 **Jackson RS**, Creemers JW, Farooqi IS, Raffin-Sanson ML, Varro A, Dockray GJ, Holst JJ, Brubaker PL, Corvol P, Polonsky KS, Ostrega D, Becker KL, Bertagna X, Hutton JC, White A, Dattani MT, Hussain K, Middleton SJ, Nicole TM, Milla PJ, Lindley KJ, O'Rahilly S. Small-intestinal dysfunction accompanies the complex endocrinopathy of human proprotein convertase 1 deficiency. *J Clin Invest* 2003; **112**: 1550-1560 [PMID: 14617756 DOI: 10.1172/JCI18784]
- 144 **Jackson RS**, Creemers JW, Ohagi S, Raffin-Sanson ML, Sanders L, Montague CT, Hutton JC, O'Rahilly S. Obesity and impaired prohormone processing associated with mutations in the human prohormone convertase 1 gene. *Nat Genet* 1997; **16**: 303-306 [PMID: 9207799 DOI: 10.1038/ng0797-303]
- 145 **Frank GR**, Fox J, Candela N, Jovanovic Z, Bochukova E, Levine J, Papenhausen PR, O'Rahilly S, Farooqi IS. Severe obesity and diabetes insipidus in a patient with PCSK1 deficiency. *Mol Genet Metab* 2013; **110**: 191-194 [PMID: 23800642 DOI: 10.1016/j.jymgme.2013.04.005]
- 146 **Bandsma RH**, Sokollik C, Chami R, Cutz E, Brubaker PL, Hamilton JK, Perlman K, Zlotkin S, Sigalek DL, Sherman PM, Martin MG, Avitzur Y. From diarrhea to obesity in prohormone convertase 1/3 deficiency: age-dependent clinical, pathologic, and enteroendocrine characteristics. *J Clin Gastroenterol* 2013; **47**: 834-843 [PMID: 24135795 DOI: 10.1097/MCG.0b013e3182a89fc8]
- 147 **Parker JA**, McCullough KA, Field BC, Minnion JS, Martin NM, Ghatei MA, Bloom SR. Glucagon and GLP-1 inhibit food intake and increase c-fos expression in similar appetite regulating centres in the brainstem and amygdala. *Int J Obes (Lond)* 2013; **37**: 1391-1398 [PMID: 23337772 DOI: 10.1038/ijo.2012.227]
- 148 **Lake A**, Townshend T. Obesogenic environments: exploring the built and food environments. *J R Soc Promot Health* 2006; **126**: 262-267 [PMID: 17152319 DOI: 10.1177/1466424006070487]
- 149 **Mackenbach JD**, Rutter H, Compennolle S, Glonti K, Oppert JM, Charreire H, De Bourdeaudhuij I, Brug J, Nijpels G, Lakerveld J. Obesogenic environments: a systematic review of the association between the physical environment and adult weight status, the SPOTLIGHT project. *BMC Public Health* 2014; **14**: 233 [PMID: 24602291 DOI: 10.1186/1471-2458-14-233]
- 150 **Mackenbach JD**, Rutter H, Compennolle S, Glonti K, Oppert JM, Charreire H, De Bourdeaudhuij I, Brug J, Nijpels G, Lakerveld J. Obesogenic environments: a systematic review of the association between the physical environment and adult weight status, the SPOTLIGHT project. *BMC Public Health* 2014; **14**: 233 [PMID: 24602291 DOI: 10.1186/1471-2458-14-233]
- 151 **Maes HH**, Neale MC, Eaves LJ. Genetic and environmental factors in relative body weight and human adiposity. *Behav Genet*



- 1997; **27**: 325-351 [PMID: 9519560]
- 152 **O'Rahilly S**, Farooqi IS. Human obesity as a heritable disorder of the central control of energy balance. *Int J Obes (Lond)* 2008; **32** Suppl 7: S55-S61 [PMID: 19136992 DOI: 10.1038/ijo.2008.239]
  - 153 **O'Rahilly S**, Farooqi IS. Human obesity: a heritable neuro-behavioral disorder that is highly sensitive to environmental conditions. *Diabetes* 2008; **57**: 2905-2910 [PMID: 18971438 DOI: 10.2337/db08-0210]
  - 154 **Hebebrand J**, Hinney A. Environmental and genetic risk factors in obesity. *Child Adolesc Psychiatr Clin N Am* 2009; **18**: 83-94 [PMID: 19014859 DOI: 10.1016/j.chc.2008.07.006]
  - 155 **Locke AE**, Kahali B, Berndt SI, Justice AE, Pers TH, Day FR, Powell C, Vedantam S, Buchkovich ML, Yang J, Croteau-Chonka DC, Esko T, Fall T, Ferreira T, Gustafsson S, Kutalik Z, Luan J, Mägi R, Randall JC, Winkler TW, Wood AR, Workalemahu T, Faul JD, Smith JA, Hua Zhao J, Zhao W, Chen J, Fehrmann R, Hedman ÅK, Karjalainen J, Schmidt EM, Absher D, Amin N, Anderson D, Beekman M, Bolton JL, Bragg-Gresham JL, Buyske S, Demirkan A, Deng G, Ehret GB, Feenstra B, Feitosa MF, Fischer K, Goel A, Gong J, Jackson AU, Kanoni S, Kleber ME, Kristiansson K, Lim U, Lotay V, Mangino M, Mateo Leach I, Medina-Gomez C, Medland SE, Nalls MA, Palmer CD, Pasko D, Pechlivanis S, Peters MJ, Prokopenko I, Shungin D, Stančáková A, Strawbridge RJ, Ju Sung Y, Tanaka T, Teumer A, Trompet S, van der Laan SW, van Setten J, Van Vliet-Ostaptchouk JV, Wang Z, Yengo L, Zhang W, Isaacs A, Albrecht E, Ärnlöv J, Arscott GM, Attwood AP, Bandinelli S, Barrett A, Bas IN, Bellis C, Bennett AJ, Berne C, Blagieva R, Blüher M, Böhringer S, Bonnycastle LL, Böttcher Y, Boyd HA, Bruinenberg M, Caspersen IH, Ida Chen YD, Clarke R, Daw EW, de Craen AJ, Delgado G, Dimitriou M, Doney AS, Eklund N, Estrada K, Eury E, Folkersen B, Fraser RM, Garcia ME, Geller F, Giedraitis V, Gigante B, Go AS, Golay A, Goodall AH, Gordon SD, Gorski M, Grabe HJ, Grallert H, Grammer TB, Gräßler J, Grönberg H, Groves CJ, Gusto G, Haessler J, Hall P, Haller T, Hallmans G, Hartman CA, Hassinen M, Hayward C, Heard-Costa NL, Helmer Q, Hengstenberg C, Holmen O, Hottenga JJ, James AL, Jeff JM, Johansson Å, Jolley J, Juliusdottir T, Kinnunen L, Koenig W, Koskenvuo M, Kratzer W, Laitinen J, Lamina C, Leander K, Lee NR, Lichtner P, Lind L, Lindström J, Sin Lo K, Lobbens S, Lohrbeier R, Lu Y, Mach F, Magnusson PK, Mahajan A, McArdle WL, McLachlan S, Menni C, Merger S, Mihailov E, Milani L, Moayyeri A, Monda KL, Morken MA, Mulas A, Müller G, Müller-Nurasyid M, Musk AW, Nagaraja R, Nöthen MM, Nolte IM, Pilz S, Rayner NW, Renstrom F, Rettig R, Ried JS, Ripke S, Robertson NR, Rose LM, Sanna S, Schanagl H, Scholtens S, Schumacher FR, Scott WR, Seufferlein T, Shi J, Vernon Smith A, Smolonska J, Stanton AV, Steinthorsdottir V, Stirrups K, Stringham HM, Sundström J, Swertz MA, Swift AJ, Syvänen AC, Tan ST, Tayo BO, Thorand B, Thorleifsson G, Tyrer JP, Uh HW, Vandenput L, Verhulst FC, Vermeulen SH, Verweij N, Vonk JM, Waite LL, Warren HR, Waterworth D, Weedon MN, Wilkens LR, Willenborg C, Wilsgaard T, Wojczynski MK, Wong A, Wright AF, Zhang Q, Brennan EP, Choi M, Dastani Z, Drong AW, Eriksson P, Franco-Cereceda A, Gådin JR, Gharavi AG, Goddard ME, Handsaker RE, Huang J, Karpe F, Kathiresan S, Keildson S, Kiryluk K, Kubo M, Lee JY, Liang L, Lifton RP, Ma B, McCarroll SA, McKnight AJ, Min JL, Moffatt MF, Montgomery GW, Murabito JM, Nicholson G, Nyholt DR, Okada Y, Perry JR, Dorajoo R, Reinmaa E, Salem RM, Sandholm N, Scott RA, Stolk L, Takahashi A, Tanaka T, Van't Hooft FM, Vinkhuyzen AA, Westra HJ, Zheng W, Zondervan KT, Heath AC, Arveiler D, Bakker SJ, Beilby J, Bergman RN, Blangero J, Bovet P, Campbell H, Caulfield MJ, Cesana G, Chakravarti A, Chasman DI, Chines PS, Collins FS, Crawford DC, Cupples LA, Cusi D, Danesh J, de Faire U, den Ruijter HM, Dominiczak AF, Erbel R, Erdmann J, Eriksson JG, Farrall M, Felix SB, Ferrannini E, Ferrières J, Ford I, Frouhi NG, Forrester T, Franco OH, Gansevoort RT, Gejman PV, Gieger C, Gottesman O, Gudnason V, Gyllenstein U, Hall AS, Harris TB, Hattersley AT, Hicks AA, Hindorf LA, Hingorani AD, Hofman A, Homuth G, Hovingh GK, Humphries SE, Hunt SC, Hyppönen E, Illig T, Jacobs KB, Jarvelin MR, Jöckel KH, Johansen B, Jousilahti P, Jukema JW, Jula AM, Kaprio J, Kastelein JJ, Keinanen-Kiukkaanniemi SM, Kiemeny LA, Knekt P, Kooner JS, Kooperberg C, Kovacs P, Kraja AT, Kumari M, Kuusisto J, Lakka TA, Langenberg C, Le Marchand L, Lehtimäki T, Lyssenko V, Männistö S, Marette A, Matise TC, McKenzie CA, McKnight B, Moll FL, Morris AD, Morris AP, Murray JC, Nelis M, Ohlsson C, Oldehinkel AJ, Ong KK, Madden PA, Pasterkamp G, Peden JF, Peters A, Postma DS, Pramstaller PP, Price JF, Qi L, Raitakari OT, Rankinen T, Rao DC, Rice TK, Ridker PM, Rioux JD, Ritchie MD, Rudan I, Salomaa V, Samani NJ, Saramies J, Sarzynski MA, Schunkert H, Schwarz PE, Sever P, Shuldiner AR, Sinisalo J, Stolk RP, Strauch K, Tönjes A, Trégouët DA, Tremblay A, Tremoli E, Virtamo J, Vohl MC, Völker U, Waeber G, Willemsen G, Witteman JC, Zillikens MC, Adair LS, Amouyel P, Asselbergs FW, Assimes TL, Bochud M, Boehm BO, Boerwinkle E, Bornstein SR, Bottinger EP, Bouchard C, Cauchi S, Chambers JC, Chanock SJ, Cooper RS, de Bakker PI, Dedoussis G, Ferrucci L, Franks PW, Froguel P, Groop LC, Haiman CA, Hamsten A, Hui J, Hunter DJ, Hveem K, Kaplan RC, Kivimäki M, Kuh D, Laakso M, Liu Y, Martin NG, März W, Melbye M, Metspalu A, Moebus S, Munroe PB, Njølstad I, Oostra BA, Palmer CN, Pedersen NL, Perola M, Pérusse L, Peters U, Power C, Quertermous T, Rauramaa R, Rivadeneira F, Saaristo TE, Saleheen D, Sattar N, Schadt EE, Schlessinger D, Slagboom PE, Snieder H, Spector TD, Thorsteinsdottir U, Stumvoll M, Tuomilehto J, Uitterlinden AG, Uusitupa M, van der Harst P, Walker M, Wallaschofski H, Wareham NJ, Watkins H, Weir DR, Wichmann HE, Wilson JF, Zanen P, Borecki IB, Deloukas P, Fox CS, Heid IM, O'Connell JR, Strachan DP, Stefansson K, van Duijn CM, Abecasis GR, Franke L, Frayling TM, McCarthy MI, Visscher PM, Scherag A, Willer CJ, Boehnke M, Mohlke KL, Lindgren CM, Beckmann JS, Barroso I, North KE, Ingelsson E, Hirschhorn JN, Loos RJ, Speliotes EK. Genetic studies of body mass index yield new insights for obesity biology. *Nature* 2015; **518**: 197-206 [PMID: 25673413 DOI: 10.1038/nature14177]
  - 156 **Choquet H**, Meyre D. Genetics of Obesity: What have we Learned? *Curr Genomics* 2011; **12**: 169-179 [PMID: 22043165 DOI: 10.2174/138920211795677895]
  - 157 **Benzinou M**, Creemers JW, Choquet H, Lobbens S, Dina C, Durand E, Guerdardel A, Boutin P, Jouret B, Heude B, Balkau B, Tichet J, Marre M, Potoczna N, Horber F, Le Stunff C, Czernichow S, Sandbaek A, Lauritzen T, Borch-Johnsen K, Andersen G, Kiess W, Körner A, Kovacs P, Jacobson P, Carlsson LM, Walley AJ, Jørgensen T, Hansen T, Pedersen O, Meyre D, Froguel P. Common nonsynonymous variants in PCSK1 confer risk of obesity. *Nat Genet* 2008; **40**: 943-945 [PMID: 18604207 DOI: 10.1038/ng.177]
  - 158 **Choquet H**, Kasberger J, Hamidovic A, Jorgenson E. Contribution of common PCSK1 genetic variants to obesity in 8,359 subjects from multi-ethnic American population. *PLoS One* 2013; **8**: e57857 [PMID: 23451278 DOI: 10.1371/journal.pone.0057857]
  - 159 **Kilpeläinen TO**, Bingham SA, Khaw KT, Wareham NJ, Loos RJ. Association of variants in the PCSK1 gene with obesity in the EPIC-Norfolk study. *Hum Mol Genet* 2009; **18**: 3496-3501 [PMID: 19528091 DOI: 10.1093/hmg/ddp280]
  - 160 **Qi Q**, Li H, Loos RJ, Liu C, Hu FB, Wu H, Yu Z, Lin X. Association of PCSK1 rs6234 with obesity and related traits in a Chinese Han population. *PLoS One* 2010; **5**: e10590 [PMID: 20498726 DOI: 10.1371/journal.pone.0010590]
  - 161 **Chang YC**, Chiu YF, Shih KC, Lin MW, Sheu WH, Donlon T, Curb JD, Jou YS, Chang TJ, Li HY, Chuang LM. Common PCSK1 haplotypes are associated with obesity in the Chinese population. *Obesity* (Silver Spring) 2010; **18**: 1404-1409 [PMID: 19875984 DOI: 10.1038/oby.2009.390]
  - 162 **Ritze Y**, Hengelhaupt C, Bárdos G, Ernst B, Thurnheer M, D'Haese JG, Bischoff SC, Schultes B. Altered intestinal neuroendocrine gene expression in humans with obesity. *Obesity* (Silver Spring) 2015; **23**: 2278-2285 [PMID: 26381270 DOI: 10.1002/oby.21253]
  - 163 **Roth KA**, Kim S, Gordon JL. Immunocytochemical studies suggest two pathways for enteroendocrine cell differentiation in the colon.

- Am J Physiol* 1992; **263**: G174-G180 [PMID: 1514628]
- 164 **Gagnon J**, Baggio LL, Drucker DJ, Brubaker PL. Ghrelin Is a Novel Regulator of GLP-1 Secretion. *Diabetes* 2015; **64**: 1513-1521 [PMID: 25412624 DOI: 10.2337/db14-1176]
  - 165 **Marzullo P**, Verti B, Savia G, Walker GE, Guzzaloni G, Tagliaferri M, Di Blasio A, Liuzzi A. The relationship between active ghrelin levels and human obesity involves alterations in resting energy expenditure. *J Clin Endocrinol Metab* 2004; **89**: 936-939 [PMID: 14764817 DOI: 10.1210/jc.2003-031328]
  - 166 **Rosická M**, Krsek M, Matoulek M, Jarkovská Z, Marek J, Justová V, Lacinová Z. Serum ghrelin levels in obese patients: the relationship to serum leptin levels and soluble leptin receptors levels. *Physiol Res* 2003; **52**: 61-66 [PMID: 12625808]
  - 167 **Jeppesen PB**, Hartmann B, Thulesen J, Hansen BS, Holst JJ, Poulsen SS, Mortensen PB. Elevated plasma glucagon-like peptide 1 and 2 concentrations in ileum resected short bowel patients with a preserved colon. *Gut* 2000; **47**: 370-376 [PMID: 10940274]
  - 168 **Adam TC**, Westerterp-Plantenga MS. Glucagon-like peptide-1 release and satiety after a nutrient challenge in normal-weight and obese subjects. *Br J Nutr* 2005; **93**: 845-851 [PMID: 16022753 DOI: 10.1079/BJN20041335]
  - 169 **Verdich C**, Toubro S, Buemann B, Lysgård Madsen J, Juul Holst J, Astrup A. The role of postprandial releases of insulin and incretin hormones in meal-induced satiety--effect of obesity and weight reduction. *Int J Obes Relat Metab Disord* 2001; **25**: 1206-1214 [PMID: 11477506 DOI: 10.1038/sj.ijo.0801655]
  - 170 **Ranganath LR**, Beety JM, Morgan LM, Wright JW, Howland R, Marks V. Attenuated GLP-1 secretion in obesity: cause or consequence? *Gut* 1996; **38**: 916-919 [PMID: 8984033]
  - 171 **Færch K**, Torekov SS, Vistisen D, Johansen NB, Witte DR, Jonsson A, Pedersen O, Hansen T, Lauritzen T, Sandbæk A, Holst JJ, Jørgensen ME. GLP-1 Response to Oral Glucose Is Reduced in Prediabetes, Screen-Detected Type 2 Diabetes, and Obesity and Influenced by Sex: The ADDITION-PRO Study. *Diabetes* 2015; **64**: 2513-2525 [PMID: 25677912 DOI: 10.2337/db14-1751]
  - 172 **Hussein MS**, Abushady MM, Refaat S, Ibrahim R. Plasma level of glucagon-like peptide 1 in obese Egyptians with normal and impaired glucose tolerance. *Arch Med Res* 2014; **45**: 58-62 [PMID: 24321596 DOI: 10.1016/j.arcmed.2013.10.012]
  - 173 **Muscelli E**, Mari A, Casolaro A, Camastra S, Seghieri G, Gastaldelli A, Holst JJ, Ferrannini E. Separate impact of obesity and glucose tolerance on the incretin effect in normal subjects and type 2 diabetic patients. *Diabetes* 2008; **57**: 1340-1348 [PMID: 18162504 DOI: 10.2337/db07-1315]
  - 174 **Svendsen PF**, Jensen FK, Holst JJ, Haugaard SB, Nilas L, Madsbad S. The effect of a very low calorie diet on insulin sensitivity, beta cell function, insulin clearance, incretin hormone secretion, androgen levels and body composition in obese young women. *Scand J Clin Lab Invest* 2012; **72**: 410-419 [PMID: 22708619 DOI: 10.3109/00365513.2012.691542]
  - 175 **Carroll JF**, Kaiser KA, Franks SF, Deere C, Caffrey JL. Influence of BMI and gender on postprandial hormone responses. *Obesity* (Silver Spring) 2007; **15**: 2974-2983 [PMID: 18198306 DOI: 10.1038/oby.2007.355]
  - 176 **Vilshøj T**, Krarup T, Sonne J, Madsbad S, Vølund A, Juul AG, Holst JJ. Incretin secretion in relation to meal size and body weight in healthy subjects and people with type 1 and type 2 diabetes mellitus. *J Clin Endocrinol Metab* 2003; **88**: 2706-2713 [PMID: 12788877 DOI: 10.1210/jc.2002-021873]
  - 177 **Toft-Nielsen MB**, Damholt MB, Madsbad S, Hilsted LM, Hughes TE, Michelsen BK, Holst JJ. Determinants of the impaired secretion of glucagon-like peptide-1 in type 2 diabetic patients. *J Clin Endocrinol Metab* 2001; **86**: 3717-3723 [PMID: 11502801 DOI: 10.1210/jcem.86.8.7750]
  - 178 **Näslund E**, Grybäck P, Backman L, Jacobsson H, Holst JJ, Theodorsson E, Hellström PM. Distal small bowel hormones: correlation with fasting antroduodenal motility and gastric emptying. *Dig Dis Sci* 1998; **43**: 945-952 [PMID: 9590405]
  - 179 **McKeigue PM**, Pierpoint T, Ferrie JE, Marmot MG. Relationship of glucose intolerance and hyperinsulinaemia to body fat pattern in south Asians and Europeans. *Diabetologia* 1992; **35**: 785-791 [PMID: 1511807]
  - 180 **Mannucci E**, Ognibene A, Cremasco F, Bardini G, Mencucci A, Pierazzuoli E, Ciani S, Fanelli A, Messeri G, Rotella CM. Glucagon-like peptide (GLP)-1 and leptin concentrations in obese patients with Type 2 diabetes mellitus. *Diabet Med* 2000; **17**: 713-719 [PMID: 11110504 DOI: 10.1046/j.1464-5491.2000.00367.x]
  - 181 **Zhang Y**, Scarpace PJ. The role of leptin in leptin resistance and obesity. *Physiol Behav* 2006; **88**: 249-256 [PMID: 16782141 DOI: 10.1016/j.physbeh.2006.05.038]
  - 182 **Anini Y**, Brubaker PL. Role of leptin in the regulation of glucagon-like peptide-1 secretion. *Diabetes* 2003; **52**: 252-259 [PMID: 12540594 DOI: 10.2337/diabetes.52.2.252]
  - 183 **Bewick GA**, Kent A, Campbell D, Patterson M, Gbatei MA, Bloom SR, Gardiner JV. Mice with hyperghrelinemia are hyperphagic and glucose intolerant and have reduced leptin sensitivity. *Diabetes* 2009; **58**: 840-846 [PMID: 19151202 DOI: 10.2337/db08-1428]
  - 184 **Daghestani MH**. A preprandial and postprandial plasma levels of ghrelin hormone in lean, overweight and obese Saudi females. *Journal of King Saud University - Science* 2009; **21**: 119-124 [DOI: 10.1016/j.jksus.2009.05.001]
  - 185 **English PJ**, Gbatei MA, Malik IA, Bloom SR, Wilding JP. Food fails to suppress ghrelin levels in obese humans. *J Clin Endocrinol Metab* 2002; **87**: 2984 [PMID: 12050284 DOI: 10.1210/jcem.87.6.8738]
  - 186 **Korner J**, Inabnet W, Febres G, Conwell IM, McMahon DJ, Salas R, Taveras C, Schroppe B, Bessler M. Prospective study of gut hormone and metabolic changes after adjustable gastric banding and Roux-en-Y gastric bypass. *Int J Obes (Lond)* 2009; **33**: 786-795 [PMID: 19417773 DOI: 10.1038/ijo.2009.79]
  - 187 **Korner J**, Bessler M, Inabnet W, Taveras C, Holst JJ. Exaggerated glucagon-like peptide-1 and blunted glucose-dependent insulinotropic peptide secretion are associated with Roux-en-Y gastric bypass but not adjustable gastric banding. *Surg Obes Relat Dis* 2007; **3**: 597-601 [PMID: 17936091 DOI: 10.1016/j.soard.2007.08.004]
  - 188 **le Roux CW**, Aylwin SJ, Batterham RL, Borg CM, Coyle F, Prasad V, Shurey S, Gbatei MA, Patel AG, Bloom SR. Gut hormone profiles following bariatric surgery favor an anorectic state, facilitate weight loss, and improve metabolic parameters. *Ann Surg* 2006; **243**: 108-114 [PMID: 16371744 DOI: 10.1097/01.sla.0000183349.16877.84]
  - 189 **Kellum JM**, Kuemmerle JF, O'Dorisio TM, Rayford P, Martin D, Engle K, Wolf L, Sugerman HJ. Gastrointestinal hormone responses to meals before and after gastric bypass and vertical banded gastroplasty. *Ann Surg* 1990; **211**: 763-770; discussion 770-771 [PMID: 2192696]
  - 190 **Falkén Y**, Hellström PM, Holst JJ, Näslund E. Changes in glucose homeostasis after Roux-en-Y gastric bypass surgery for obesity at day three, two months, and one year after surgery: role of gut peptides. *J Clin Endocrinol Metab* 2011; **96**: 2227-2235 [PMID: 21543426 DOI: 10.1210/jc.2010-2876]
  - 191 **Borg CM**, le Roux CW, Gbatei MA, Bloom SR, Patel AG, Aylwin SJ. Progressive rise in gut hormone levels after Roux-en-Y gastric bypass suggests gut adaptation and explains altered satiety. *Br J Surg* 2006; **93**: 210-215 [PMID: 16392104 DOI: 10.1002/bjs.5227]
  - 192 **Morínigo R**, Moizé V, Musri M, Lacy AM, Navarro S, Marín JL, Delgado S, Casamitjana R, Vidal J. Glucagon-like peptide-1, peptide YY, hunger, and satiety after gastric bypass surgery in morbidly obese subjects. *J Clin Endocrinol Metab* 2006; **91**: 1735-1740 [PMID: 16478824 DOI: 10.1210/jc.2005-0904]
  - 193 **Maggard MA**, Shugarman LR, Suttrop M, Maglione M, Sugerman HJ, Sugarman HJ, Livingston EH, Nguyen NT, Li Z, Mojica WA, Hilton L, Rhodes S, Morton SC, Shekelle PG. Meta-analysis: surgical treatment of obesity. *Ann Intern Med* 2005; **142**: 547-559 [PMID: 15809466]
  - 194 **Sarson DL**, Scopinaro N, Bloom SR. Gut hormone changes after jejunoileal (JIB) or biliopancreatic (BPP) bypass surgery for morbid obesity. *Int J Obes* 1981; **5**: 471-480 [PMID: 6796532]
  - 195 **Holst JJ**, Sørensen TI, Andersen AN, Stadil F, Andersen B, Lauritsen KB, Klein HC. Plasma enteroglucagon after jejunoileal

- bypass with 3: 1 or 1: 3 jejunoileal ratio. *Scand J Gastroenterol* 1979; **14**: 205-207 [PMID: 432544 DOI: 10.3109/00365527909179871]
- 196 **Buchan AM**, Pederson RA, Koop I, Gourlay RH, Cleator IG. Morphological and functional alterations to a sub-group of regulatory peptides in human pancreas and intestine after jejuno-ileal bypass. *Int J Obes Relat Metab Disord* 1993; **17**: 109-113 [PMID: 8095927]
- 197 **Stice E**, Spoor S, Bohon C, Small DM. Relation between obesity and blunted striatal response to food is moderated by TaqIA A1 allele. *Science* 2008; **322**: 449-452 [PMID: 18927395 DOI: 10.1126/science.1161550]
- 198 **Stice E**, Spoor S, Bohon C, Veldhuizen MG, Small DM. Relation of reward from food intake and anticipated food intake to obesity: a functional magnetic resonance imaging study. *J Abnorm Psychol* 2008; **117**: 924-935 [PMID: 19025237 DOI: 10.1037/a0013600]
- 199 **van Bloemendaal L**, Veltman DJ, Ten Kulve JS, Groot PF, Ruhé HG, Barkhof F, Sloan JH, Diamant M, Ijzerman RG. Brain reward-system activation in response to anticipation and consumption of palatable food is altered by glucagon-like peptide-1 receptor activation in humans. *Diabetes Obes Metab* 2015; **17**: 878-886 [PMID: 26094857 DOI: 10.1111/dom.12506]
- 200 **van Bloemendaal L**, Ijzerman RG, Ten Kulve JS, Barkhof F, Konrad RJ, Drent ML, Veltman DJ, Diamant M. GLP-1 receptor activation modulates appetite- and reward-related brain areas in humans. *Diabetes* 2014; **63**: 4186-4196 [PMID: 25071023 DOI: 10.2337/db14-0849]
- 201 **Witt AA**, Raggio GA, Butryn ML, Lowe MR. Do hunger and exposure to food affect scores on a measure of hedonic hunger? An experimental study. *Appetite* 2014; **74**: 1-5 [PMID: 24269255 DOI: 10.1016/j.appet.2013.11.010]
- 202 **Singh M**. Mood, food, and obesity. *Front Psychol* 2014; **5**: 925 [PMID: 25225489 DOI: 10.3389/fpsyg.2014.00925]
- 203 **van Bloemendaal L**, Veltman DJ, ten Kulve JS, Drent ML, Barkhof F, Diamant M, Ijzerman RG. Emotional eating is associated with increased brain responses to food-cues and reduced sensitivity to GLP-1 receptor activation. *Obesity* (Silver Spring) 2015; **23**: 2075-2082 [PMID: 26331843 DOI: 10.1002/oby.21200]
- 204 **Scott LJ**. Liraglutide: a review of its use in the management of obesity. *Drugs* 2015; **75**: 899-910 [PMID: 25985864 DOI: 10.1007/s40265-015-0408-8]
- 205 **Pi-Sunyer X**, Astrup A, Fujioka K, Greenway F, Halpern A, Krempf M, Lau DC, le Roux CW, Violante Ortiz R, Jensen CB, Wilding JP. A Randomized, Controlled Trial of 3.0 mg of Liraglutide in Weight Management. *N Engl J Med* 2015; **373**: 11-22 [PMID: 26132939 DOI: 10.1056/NEJMoa1411892]
- 206 **Wadden TA**, Hollander P, Klein S, Niswender K, Woo V, Hale PM, Aronne L. Weight maintenance and additional weight loss with liraglutide after low-calorie-diet-induced weight loss: the SCALE Maintenance randomized study. *Int J Obes (Lond)* 2013; **37**: 1443-1451 [PMID: 23812094 DOI: 10.1038/ijo.2013.120]
- 207 **Davies MJ**, Bergenstal R, Bode B, Kushner RF, Lewin A, Skjoth TV, Andreasen AH, Jensen CB, DeFronzo RA. Efficacy of Liraglutide for Weight Loss Among Patients With Type 2 Diabetes: The SCALE Diabetes Randomized Clinical Trial. *JAMA* 2015; **314**: 687-699 [PMID: 26284720 DOI: 10.1001/jama.2015.9676]
- 208 **Kenny PJ**. Reward mechanisms in obesity: new insights and future directions. *Neuron* 2011; **69**: 664-679 [PMID: 21338878 DOI: 10.1016/j.neuron.2011.02.016]
- 209 **van Can J**, Sloth B, Jensen CB, Flint A, Blaak EE, Saris WH. Effects of the once-daily GLP-1 analog liraglutide on gastric emptying, glycemic parameters, appetite and energy metabolism in obese, non-diabetic adults. *Int J Obes (Lond)* 2014; **38**: 784-793 [PMID: 23999198 DOI: 10.1038/ijo.2013.162]
- 210 **Vallöf D**, Maccioni P, Colombo G, Mandrup M, Jörnulf JW, Eggecioglu E, Engel JA, Jerlhag E. The glucagon-like peptide 1 receptor agonist liraglutide attenuates the reinforcing properties of alcohol in rodents. *Addict Biol* 2016; **21**: 422-437 [PMID: 26303264 DOI: 10.1111/adb.12295]
- 211 **Bray GA**. Why do we need drugs to treat the patient with obesity? *Obesity* (Silver Spring) 2013; **21**: 893-899 [PMID: 23520198 DOI: 10.1002/oby.20394]
- 212 **Pi-Sunyer FX**, Astrup A, Fujioka K, Greenway FL, Halpern A, Krempf M, Lau DC, le Roux C, Ortiz RV, Jensen CB, Wilding J. Liraglutide 3.0 Mg Reduces the Prevalence of Prediabetes and Delays Onset of Type 2 Diabetes in Overweight and Obese Adults: Results from Scale Obesity and Prediabetes, a Randomized, Double-Blind and Placebo-Controlled 56-Week Trial. 2014. Available from: URL: <http://press.endocrine.org/doi/abs/10.1210/endo-meetings.2014.OABA.19.PP05-4#sthash.adiubGqI.dpuf>
- 213 **Public Health England**. Adult obesity and type 2 diabetes. 2014: p6-p17. [accessed 2015 Nov 17]. Available from: URL: [https://www.gov.uk/government/uploads/system/uploads/attachment\\_data/file/338934/Adult\\_obesity\\_and\\_type\\_2\\_diabetes.pdf](https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/338934/Adult_obesity_and_type_2_diabetes.pdf)
- 214 **Mannucci E**, Ognibene A, Cremasco F, Bardini G, Mencucci A, Pierazzuoli E, Ciani S, Messeri G, Rotella CM. Effect of metformin on glucagon-like peptide 1 (GLP-1) and leptin levels in obese nondiabetic subjects. *Diabetes Care* 2001; **24**: 489-494 [PMID: 11289473 DOI: 10.2337/diacare.24.3.489]
- 215 **Campbell IW**, Howlett HC. Worldwide experience of metformin as an effective glucose-lowering agent: a meta-analysis. *Diabetes Metab Rev* 1995; **11** Suppl 1: S57-S62 [PMID: 8529486 DOI: 10.1002/dmr.5610110509]
- 216 **Hex N**, Bartlett C, Wright D, Taylor M, Varley D. Estimating the current and future costs of Type 1 and Type 2 diabetes in the UK, including direct health costs and indirect societal and productivity costs. *Diabet Med* 2012; **29**: 855-862 [PMID: 22537247 DOI: 10.1111/j.1464-5491.2012.03698.x]
- 217 **Novo Nordisk**. Saxenda; injectable liraglutide for obesity. Drugs information leaflet ONLINE. Revised 2015. Available from: URL: <https://www.saxenda.com/>
- 218 **European Medicines Agency**. Assessment report: Saxenda. 2015. Available from: URL: [http://www.ema.europa.eu/docs/en\\_GB/document\\_library/EPAR\\_-\\_Public\\_assessment\\_report/human/003780/WC500185788.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/003780/WC500185788.pdf)
- 219 **Haluzík M**, Mráz M, Svačina Š. Balancing benefits and risks in patients receiving incretin-based therapies: focus on cardiovascular and pancreatic side effects. *Drug Saf* 2014; **37**: 1003-1010 [PMID: 25391858 DOI: 10.1007/s40264-014-0238-8]
- 220 **Nauck MA**, Friedrich N. Do GLP-1-based therapies increase cancer risk? *Diabetes Care* 2013; **36** Suppl 2: S245-S252 [PMID: 23882053 DOI: 10.2337/dcS13-2004]

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## Place of technosphere inhaled insulin in treatment of diabetes

Nasser Mikhail

Nasser Mikhail, Department of Medicine, OliveView-UCLA Medical Center, Sylmar, CA 91342, United States

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Correspondence to: Nasser Mikhail, MD, Department of Medicine, OliveView-UCLA Medical Center, 14445 Olive View Dr, Sylmar, CA 91342, United States. [nmikhail@dhs.lacounty.gov](mailto:nmikhail@dhs.lacounty.gov)  
Telephone: +1-818-3643205  
Fax: +1-818-3644573

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### Abstract

Technosphere insulin (TI), Afrezza, is a powder form of short-acting regular insulin taken by oral inhalation with meals. Action of TI peaks after approximately 40-60 min and lasts for 2-3 h. TI is slightly less effective than subcutaneous insulin aspart, with mean hemoglobin A1c (HbA1c) reduction of 0.21% and 0.4%, respectively. When compared with technosphere inhaled placebo, the decrease in HbA1c levels was 0.8% and 0.4% with

TI and placebo, respectively. Compared with insulin aspart, TI is associated with lower risk of late post-prandial hypoglycemia and weight gain. Apart from hypoglycemia, cough is the most common adverse effect of TI reported by 24%-33% of patients vs 2% with insulin aspart. TI is contraindicated in patients with asthma and chronic obstructive pulmonary disease. While TI is an attractive option of prandial insulin, its use is limited by frequent occurrence of cough, need for periodic monitoring of pulmonary function, and lack of long-term safety data. Candidates for use of TI are patients having frequent hypoglycemia while using short-acting subcutaneous insulin, particularly late post-prandial hypoglycemia, patients with needle phobia, and those who cannot tolerate subcutaneous insulin due to skin reactions.

**Key words:** Afrezza; Efficacy; Safety; Technosphere insulin; Cough

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**Core tip:** Technosphere insulin is the only approved form of inhaled insulin. It is a short-acting insulin that can be taken with meals in patients with type 1 or type 2 diabetes. In this minireview, the author provides an appraisal of this new formulation of insulin to help determine its place in the management of diabetes.

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### INTRODUCTION

In June 2014, the Food and Drug Administration (FDA) approved technosphere insulin (TI) under the trade name Afrezza (MannKind Corp., Valencia, CA) for use in



type 1 and type 2 diabetes<sup>[1]</sup>. Afrezza is a dry powder of recombinant human insulin adsorbed onto an inert excipient of fumaryl diketopiperazine (FDKP) particles<sup>[2]</sup>. In this powder, insulin and FDKP are present in a 1:9 ratio by dry weight<sup>[3]</sup>. The median aerodynamic diameter of technosphere microparticles is 2-2.5  $\mu\text{m}$ , suited for deposition in distal lungs<sup>[3]</sup>. In initial studies, Afrezza was delivered by a device called MedTone. In 2010, the manufacturer produced a smaller and more efficient device called Gen2<sup>[4]</sup>. The main purpose of this article is to identify the best candidates for the use TI based on its pharmacodynamics, efficacy and safety. More emphasis will be placed on data derived from clinical trials using the current Gen2 device of TI.

## SEARCH METHODOLOGY

PubMed search was conducted until July 2016 to identify all humans studies related to efficacy and safety of TI published in the English, Spanish and French literature. The search included pertinent animal and *in-vitro* studies. Review articles, and prescribing information of afrezza are also reviewed. Search terms included "inhaled insulin", "diabetes mellitus", "technosphere", "pulmonary safety", "cough", "lung cancer".

## ABSORPTION AND METABOLISM

Upon inhalation, a mean of approximately 60% of the emitted dose of TI reaches the lungs. The remainder 40% is swallowed and enters the gastrointestinal tract<sup>[3]</sup>. The technosphere particles dissolve rapidly at the physiological pH of the lungs. This dissolution allows both insulin and the excipient FDKP to be independently absorbed across the alveolar wall to the systemic circulation with a time to maximum concentration (Tmax) of 15 and 10 min, respectively<sup>[1,3]</sup>. The inhaled insulin and its carrier FDKP are rapidly cleared from the lungs. Thus, clearance half-life of insulin and FDKP from the bronchoalveolar lavage is approximately 1 h. By 12 h post-inhalation, their concentrations in the bronchoalveolar lavage are below or near the limit of quantification<sup>[3]</sup>. After systemic absorption, the carrier FDKP is excreted unchanged in urine without any evidence of pharmacological activity<sup>[5]</sup>.

## PHARMACOKINETICS AND PHARMACODYNAMICS

Bioavailability of TI (calculated based on actual insulin content of the TI cartridge) is approximately 20%-27% relative to subcutaneous regular insulin, and 33% relative to lispro<sup>[6]</sup>. TI has rapid onset of action characterized by a sharp rise in serum concentrations reaching peak levels after 12-15 min<sup>[7]</sup>. The median time to maximum effect in 12 patients with type 1 DM was shown to be 53 min (SD 74 min) compared to approximately 90-120 min with insulin lispro<sup>[7]</sup>. In another study of 11 healthy volunteers, mean time for maximal effect of TI was 42-58 min for doses 25-100 units compared to 171 min with subcutaneous regular insulin given in a single dose of 10 units<sup>[8]</sup>.

Duration of action of TI is short and fades away by approximately 160 min or 2-3 h<sup>[7,9]</sup>. Median terminal half-life of TI is 28-39 min (for doses 4-32 units) vs 145 min for subcutaneous regular insulin (15 units)<sup>[7]</sup>. Based on the above pharmacodynamics, TI is considered an ultra-rapid insulin given at the start of a meal or within 20 min after starting a meal<sup>[10]</sup>.

## EFFICACY OF TI

### Effect on blood glucose concentrations

Reduction in post-prandial hyperglycemia is the main target action of TI. In one placebo-controlled trial of patients with type 2 diabetes, patients randomized to TI had 43% more reduction in maximal postprandial glucose levels compared with inhaled placebo<sup>[11]</sup>. The study of Rosenstock *et al*<sup>[12]</sup> shed the light on timing of action of TI. Thus, compared with biaspart insulin injected 15 min before meals, the inhalation of TI within 90 s of meal ingestion was associated with significant decrease in self-monitored blood glucose (SMBG) at 1 h after meals (171 mg/dL vs 209 mg/dL with biaspart). Yet, 2 h after meals, blood glucose concentrations were similar between biaspart and TI, and after 2 h, postprandial glucose levels became higher in patients randomized to TI than biaspart<sup>[12]</sup>. The previous finding is in agreement with pharmacodynamics studies described above showing that peak action of TI occurs at approximately 1 h and fades away after 2 h<sup>[6,7]</sup>.

### Effect on hemoglobin A1c levels

Overall, available data suggest that TI is slightly less effective than subcutaneous insulin. In one meta-analysis of 8 clinical trials of type 1 and type 2 diabetes, mean hemoglobin A1c (HbA1c) reduction with subcutaneous insulin was slightly greater than TI, with net statistically significant difference of 0.16% (95%CI: 0.06%-0.25%)<sup>[13]</sup>. The same meta-analysis showed that mean HbA1c reduction compared to baseline was 0.55% (95%CI: 0.34%-0.78%) based on data compiled from 12 clinical trials using the older MedTone device and the current Gen-2 device<sup>[13]</sup>. The efficacy of the TI Gen2 device was published in 2 trials summarized in Table 1<sup>[9,10]</sup>. In one trial, the mean reduction in HbA1c levels among patients randomized to TI and aspart was 0.21% and 0.4%, respectively with a significant difference of 0.19% (95%CI: 0.02-0.36)<sup>[10]</sup>. In the second study, TI was superior to placebo, as expected, with HbA1c reduction of 0.8% vs 0.4%, respectively<sup>[9]</sup>. Although the MedTone device is no longer used, 2 randomized trials using this older device are presented in Table 2 because of their large size, and relatively long-duration (1-2 years)<sup>[12,14]</sup>. Studies that directly compare the efficacy of the 2 devices are not available. However, their short-term safety was compared in single head to head trial discussed in the safety section below<sup>[10]</sup>.

## SAFETY PROFILE OF TI

### Hypoglycemia

In clinical trials of TI-Gen 2, non-severe hypoglycemia

**Table 1 Clinical trials of technosphere insulin using Gen2 device**

Ref.	Rosenstock <i>et al</i> <sup>[9]</sup>	Bode <i>et al</i> <sup>[10]</sup>
Design	Randomized, double-blind, placebo-controlled, 24 wk-duration	Randomized, open-label, 24 wk-duration
Type of diabetes	Type 2	Type 1
Intervention	TI ( <i>n</i> = 177) <i>vs</i> placebo ( <i>n</i> = 177), both groups were on oral agents	TI ( <i>n</i> = 174) <i>vs</i> prandial aspart ( <i>n</i> = 170). Both groups received basal insulin (NPH or detemir, or glargine)
Mean HbA1c levels at baseline	8.26%	7.93%
Reduction in HbA1c <i>vs</i> baseline	-0.8% with TI and -0.4% with placebo	-0.21% with TI <i>vs</i> -0.4% with aspart
Reduction in mean HbA1c with TI <i>vs</i> comparator	-0.4% <i>vs</i> placebo (95%CI: -0.57 to -0.23)	0.19% <i>vs</i> aspart (95%CI: 0.02 to 0.36)
Proportions of patients reaching HbA1c ≤ 7%	38% with TI <i>vs</i> 19% with placebo ( <i>P</i> = 0.0005)	18% with TI <i>vs</i> 31% with aspart ( <i>P</i> = 0.01)
Proportions reporting adverse effects	61% TI <i>vs</i> 51.1% placebo	58% TI <i>vs</i> 43% aspart
Proportions of patients reporting hypoglycemia	67.8% TI <i>vs</i> 30.7% placebo ( <i>P</i> < 0.0001)	96% TI <i>vs</i> 99.4% aspart ( <i>P</i> = 0.06)
Proportions of patients reporting cough	23.7% TI <i>vs</i> 19.9% placebo (difference not statistically significant)	31.6% TI <i>vs</i> 2.3% aspart <i>P</i> < 0.05
Withdrawal due to cough	1.1% with TI <i>vs</i> 3.4% with placebo	5.7% with TI <i>vs</i> 0% with aspart
Change in mean weight	+ 0.5 kg TI <i>vs</i> -1.1 kg placebo ( <i>P</i> < 0.0001)	-0.4 kg with TI <i>vs</i> +0.9 kg aspart ( <i>P</i> = 0.01)
Change in mean FEV1 (L)	- 0.13 L with TI <i>vs</i> -0.04 L with placebo	-0.07 L with TI <i>vs</i> -0.04 L with aspart
Withdrawal due to adverse effects	4% with TI <i>vs</i> 5.1% placebo	9.2% with TI <i>vs</i> 0% aspart

FEV1: Forced expiratory volume in 1 s; TI: Technosphere insulin; HbA1c: Hemoglobin A1c.

**Table 2 Clinical trials of technosphere insulin using the Med-Tone device**

Ref.	Raskin <i>et al</i> <sup>[14]</sup>	Rosensens <i>et al</i> <sup>[12]</sup>
Design	Randomized, open label, 2 yr-duration, pulmonary safety trial	Randomized, open-label, 52-wk duration
Type of diabetes	Types 1 and 2	Type 2
Groups of subjects and intervention	TI ( <i>n</i> = 938), usual diabetes care ( <i>n</i> = 951), control subjects without diabetes ( <i>n</i> = 164)	Glargine qhs + prandial TI ( <i>n</i> = 334) <i>vs</i> <sup>1</sup> biaspart insulin bid ( <i>n</i> = 343)
Proportions of patients with adverse effects	79% TI <i>vs</i> 71% usual care	84% TI <i>vs</i> 89% biaspart
Mean HbA1c at baseline	8.7%	8.7%
Reduction in HbA1c <i>vs</i> baseline	-0.59% with TI and -0.50% with usual care	-0.68% with TI/glargine <i>vs</i> -0.76% with biaspart
Reduction in HbA1c with TI <i>vs</i> comparator	0.09% (not significant)	0.07% (not significant)
Proportions of patients reporting hypoglycemia	39.5% TI <i>vs</i> 39.1% usual care	48% glargine/TI <i>vs</i> 69% biaspart. OR 0.4 (95%CI: 0.3-0.5)
Proportions of patients reporting cough	27.8% TI <i>vs</i> 4.4% usual care	33% glargine/TI <i>vs</i> 6% biaspart
Withdrawal due to cough	4.7% TI <i>vs</i> 0% usual care	2% glargine/TI <i>vs</i> 0% biaspart
Change in mean weight	Not reported	+ 0.9 kg glargine/TI <i>vs</i> +2.5 kg biaspart. Mean difference 1.6 kg (95%CI: -2.4 to -0.7)
Decline in mean FEV1 (liters)	More decline in TI group <i>vs</i> usual care. Mean difference 0.037 (95%CI: 0.017-0.06)	-0.13 glargine/TI <i>vs</i> -0.09 biaspart (difference not significant)
Withdrawal due to adverse effects	11% TI <i>vs</i> 0.6% usual care	9% glargine/TI <i>vs</i> 4% biaspart

<sup>1</sup>Biaspart insulin is pre-mixed insulin composed of 70% insulin protamine suspension + 30% insulin aspart. FEV1: Forced expiratory volume in 1 s; TI: Technosphere insulin; HbA1c: Hemoglobin A1c.

was defined as SMBG < 70 mg/dL and/or presence of symptoms of hypoglycemia, whereas severe hypoglycemia was an event that required assistance of another person<sup>[9,10]</sup>. Compared with insulin aspart, incidence of all hypoglycemia was numerically lower in patients randomized to TI-Gen2, 96.0% and 99.4%, respectively (*P* = 0.062), and incidence of severe hypoglycemia was significantly lower with TI Gen2-treatment than with aspart, 18.4% and 29.2%, respectively<sup>[10]</sup>. Importantly, the timing of hypoglycemic events reported in patients treated with TI was consistent with its short duration of action. Hence, hypoglycemic event rates (events/patient-months) within 2 h after meals were similar in patients randomized to TI and insulin aspart. Meanwhile, 2-5 h after meals, those rates were 2-3 times less with the use of TI compared with insulin aspart<sup>[10]</sup>. On the other hand, when compared with inhaled placebo, the incidence of all

hypoglycemia was higher with TI-Gen 2 therapy (67.8%) compared with placebo (30.7%), (*P* < 0.0001), and incidence of severe hypoglycemia was 5.1% with TI *vs* 1.7% with placebo (*P* = 0.09)<sup>[9]</sup>.

### Cough and throat symptoms

Cough is the most common non-hypoglycemic adverse effect of TI reported by 24%-33% of patients randomized to TI compared to 2%-6% of patients randomized to subcutaneous insulin or usual diabetes care<sup>[9,10,13,14]</sup>. Cough induced by TI is characterized by several features. First, it is generally mild, described as severe in approximately 1% of patients<sup>[10]</sup>; second, it occurs within 10 min of inhalation<sup>[9]</sup>; third, the percentage of patients reporting cough is highest in the first week after treatment, then decreases gradually with time<sup>[10]</sup>; fourth, cough is reversible, and resolves within 1-2 d after drug discon-

tinuation<sup>[9]</sup>; fifth, the occurrence of cough did not seem to be related to changes in pulmonary function as discussed below<sup>[14]</sup>; sixth, proportions of patients who reported cough was slightly higher in patients taking TI vs technosphere inhaled placebo powder: 23.7% (42 of 177) vs 19.9% (35 of 176), respectively<sup>[9]</sup>. The latter observation suggests that cough is mainly due to the inhaled excipient powder (FDKP), and that the insulin component contributes to a lesser extent to the development of cough. The exact mechanism of cough is unclear, and is probably due to stimulation of cough reflex by dry powder inhalation<sup>[9]</sup>. Unfortunately, frequency of cough associated with the use of the current Gen2 device is markedly greater than the older MedTone device, 31.6% and 22.5%, respectively<sup>[9]</sup>. This finding was attributed to the high amount of powder being inhaled in a single inhalation with Gen2, whereas with MedTone, the amount of powder inhaled per dose was distributed over 2 inhalations<sup>[9]</sup>.

Throat pain or irritation occurred in 4.4% of patients with type 2 DM ( $n = 1991$ ) compared with 0.9% of patients using comparator (non-inhaled) therapy ( $n = 1363$ )<sup>[7]</sup>.

### **Effect of TI on pulmonary function tests**

The effect of TI delivered by MedTone device on pulmonary function was studied in a large randomized trial composed of 3 groups of subjects followed for 2 years: Patients with type 1 or type 2 diabetes receiving TI ( $n = 730$ ), patients with type 1 or type 2 diabetes receiving usual care ( $n = 824$ ), and a smaller group of subjects without diabetes not taking any medications ( $n = 145$ ) (Table 2)<sup>[14]</sup>. After 3 mo, the authors recorded an initial decline among the 3 patient groups in all parameters of pulmonary functions studied including the forced expiratory volume in 1 second (FEV1) with the largest decline occurring in the TI-treated group. The difference in decline in FEV1 from baseline to 24 mo between the TI-treated group and usual care group was small but statistically significant: 0.037 liters (95%CI: 0.014 to 0.060)<sup>[14]</sup>. However, after 3 mo, the rate of change in respiratory parameters was not statistically different between patient groups. This suggests that worsening of pulmonary function in patients treated with TI occurred early in the first 3 month, and do not progress further up to 2 years of follow-up. The manufacturer recommends that pulmonary function tests (e.g., spirometry) should be assessed before treatment initiation, after 6 mo of therapy and annually thereafter<sup>[7]</sup>. If there is reduction of 20% or more in FEV1 compared to pre-treatment values, consideration should be given for drug-discontinuation<sup>[7]</sup>. Although Raskin *et al*<sup>[14]</sup> did not found a relationship between the changes in pulmonary function and the occurrence of cough, the manufacturer recommends more frequent monitoring of pulmonary function in patients with any pulmonary symptoms such as persistent cough, wheezing and breathing difficulties, and to discontinue the drug if symptoms persist<sup>[7]</sup>. Available data are insufficient regarding reversibility of pulmonary function abnormalities after discontinuation of long-term use of TI<sup>[7]</sup>. However, in a 24-wk trial, the

authors documented reversibility of FEV1 4 wk after discontinuation of TI<sup>[10]</sup>. The exact mechanisms of decline in pulmonary function after TI inhalation are unclear. Animal studies showed that the previous form of inhaled insulin (Exubera) forms amyloid aggregates in lungs of mice and may induce mitochondrial dysfunction leading to a significant reduction in pulmonary air flow<sup>[15]</sup>.

### **Effect on weight**

The effect of TI on weight gain was less pronounced compared with subcutaneous insulin formulations. Thus, when TI was compared with twice daily premixed bipart, mean weight gain after 52 wk was 0.9 kg and 2.5 kg, respectively<sup>[12]</sup>. Moreover, the use of TI was associated with mean weight loss of 0.4 kg as opposed to a mean weight gain of 0.9 kg among patient randomized to prandial insulin aspart<sup>[10]</sup>. One meta-analysis has shown that TI was associated with less weight gain than subcutaneous insulin with a net difference of -1.1 kg (95%CI: -2.1 to -1.6 kg)<sup>[14]</sup>. Meanwhile, an average weight gain of 0.5 kg was recorded in patients randomized to TI vs a weight loss of 1.1 kg in patients randomized to placebo<sup>[9]</sup>. The reasons for low propensity of TI to cause weight gain are not entirely clear. Possible causes include its somewhat inferior efficacy and lower risk of causing late post-prandial hypoglycemia compared to subcutaneous insulin. The latter advantage might lead some patients to avoid "overeating" in an attempt to prevent hypoglycemia.

### **Diabetic ketoacidosis**

In clinical trials of TI, no reports of ketoacidosis were reported<sup>[9-12,14]</sup>. However, a meta-analysis that examined regulatory documents reported a nearly 5 times higher incidence of DKA among patients treated with TI compared with prandial short-acting insulin<sup>[13]</sup>. Likewise, the manufacturer reports higher frequency of DKA in trials of type 1 diabetes among patients using TI vs subjects receiving comparators: 0.43% ( $n = 13$ ) and 0.14% ( $n = 3$ ), respectively<sup>[7]</sup>. The reasons for this increase in DKA with TI are not understood, but could be partly attributed to its ultra-short duration of action of TI creating times of day with relative insulin deficiency.

### **Lung cancer**

In patients exposed to TI in clinical trials, the manufacturer reported 2 cases of lung cancer (2 cases in 2750 patient-years of exposure) both having prior history of heavy tobacco abuse<sup>[7]</sup>. Two other cases (both squamous cell carcinoma) occurred in non-smokers after clinical trial completion. Thus, 4 cases of lung cancer were reported in patients exposed to TI vs none in control group<sup>[7]</sup>. Although the number of affected patients is too small to draw a valid conclusion, lung cancer is certainly a major concern of inhaled insulin, particularly that lung cancer rates were found to be increased in association with the previous inhaled insulin Exubera<sup>[16]</sup>. The long-term local effects of TI and its carrier on pulmonary cell are unknown. *In vitro* studies of lung cell line (Calu-3) showed that TI did not affect insulin transport, cell viability, and plasma membrane integrity<sup>[17]</sup>. Meanwhile,

**Table 3 Candidate patients for technosphere insulin**

Patients with type 1 diabetes who are taking basal insulin once daily, but prefers to take their prandial insulin in the inhaled formulation
Patients with type 2 diabetes uncontrolled on oral agents, and are reluctant to start subcutaneous insulin due to needle phobia or other reasons
Patients already on subcutaneous prandial insulin who develop frequent late post-prandial hypoglycemia (4-5 h after meals)
Any patient who develops skin reactions to insulin subcutaneous injections such as lipoatrophy or lipohypertrophy
In combination of automated artificial pancreas to provide rapid insulin delivery right after meals <sup>[20]</sup>

**Table 4 Advantages and limitations of technosphere insulin**

Advantages
Relatively easy and non-painful administration
Flexible timing of administration either inhaled directly before meals or within 20 min after finishing a meal <sup>[10]</sup>
Hypoglycemia is less frequent than subcutaneous insulin, particularly late postprandial hypoglycemia
Weight gain is slightly less pronounced than subcutaneous insulin
Limitations
Frequent cough (24%-33% of patients)
Available only as prandial short-acting insulin. Hence, long-acting basal subcutaneous insulin should be added in patients with type 1 diabetes
Slightly less effective than subcutaneous insulin
Need for baseline and then serial pulmonary function testing
Safer to switch to subcutaneous insulin in case of upper or lower respiratory infections to avoid exacerbation of the disease and possible unreliable pulmonary absorption
No data available for pediatric and pregnant populations
Limited strength options and difficult fine titration of doses
Lack of long-term safety data
High cost, e.g., average price of ninety 4-unit cartridges and 2 inhalers is \$271 <sup>[21]</sup>

insulin is a growth factor that binds to insulin receptors and if present in high concentrations, can bind to IGF-I receptors in lungs. This binding could potentially induce new-onset pulmonary cancer or accelerate growth of pre-existing malignant cells. Indeed, isolated human bronchial carcinoma cells (H292) were shown to express insulin receptors 4-5 times higher than normal bronchial epithelial cells<sup>[18]</sup>. In fact, the FDA requested the manufacturer to conduct a clinical trial with sufficient power to examine this issue.

#### **Patients with pulmonary diseases and smokers**

TI is contraindicated in any chronic pulmonary disease such as asthma or chronic obstructive pulmonary disease (COPD)<sup>[7]</sup>. Indeed, acute bronchospasm and wheezing were observed in 29% (5 of 17) of patients with asthma following inhalation of TI compared with none of 13 individuals without asthma<sup>[7]</sup>. Moreover, in asthmatic patients, a substantial mean reduction in FEV1 of 400 mL was recorded 15 min after a single dose of TI<sup>[7]</sup>. Similarly, in a small group of patients with COPD ( $n = 8$ ), a mean decline in FEV1 of 200 mL was observed 18 min after TI inhalation<sup>[7]</sup>. These acute and severe reactions to TI among patients with asthma and COPD could be the result of airway irritation upon contact with the inhaled insulin and/or the excipient. Interestingly, no significant differences in pharmacokinetics (time to maximum concentration, peak plasma insulin concentrations, and plasma insulin exposure) were found between patients with COPD and healthy subjects after a single dose of TI<sup>[19]</sup>. In case of common cold or flu, some workers recommend switching to subcutaneous insulin until the disease resolves<sup>[4]</sup>. It is not recommended that patients who smoke use TI<sup>[7]</sup>.

## **PLACE OF INHALED INSULIN TI IN DIABETES THERAPY**

Based on available data, the use of TI is most appropriate in the following selected groups of patients (Table 3). First, patients with type 1 diabetes who are taking basal insulin once daily, but prefers to take their prandial insulin in the inhaled formulation; second, patients with type 2 diabetes uncontrolled on oral agents, and are reluctant to start subcutaneous insulin due to needle phobia or other reasons; third, patients already on subcutaneous prandial insulin who develop frequent late post-prandial hypoglycemia (4-5 h after meals); fourth, patients who develop skin reactions to insulin subcutaneous injections such as lipoatrophy or lipohypertrophy. Another potential place of TI that is under investigations includes its use in combination of automated artificial pancreas to provide rapid insulin delivery right after meals<sup>[20]</sup>.

## **CONCLUSION**

Despite its limitations, TI represents a useful addition to the treatment of diabetes. Its easy non-invasive way of administration is a major advantage to patients who do not like injections. Although TI is slightly less effective than the subcutaneous insulin analog aspart, this is balanced by its lower risk of causing late postprandial hypoglycemia and weight gain. Cough remains a major limiting factor of TI occurring mainly in early treatment. Long-term clinical trials of adequate power along with post-marketing (phase IV) studies are needed to clarify the long-term safety of TI and its relationship to lung cancer. Advantages and limitations of TI are summarized in Table 4.



## REFERENCES

- 1 **Klonoff DC.** Afrezza inhaled insulin: the fastest-acting FDA-approved insulin on the market has favorable properties. *J Diabetes Sci Technol* 2014; **8**: 1071-1073 [PMID: 25355710 DOI: 10.1177/1932296814555820]
- 2 **Nuffer W,** Trujillo JM, Ellis SL. Technosphere insulin (Afrezza): a new, inhaled prandial insulin. *Ann Pharmacother* 2015; **49**: 99-106 [PMID: 25313261 DOI: 10.1177/1060028014554648]
- 3 **Cassidy JP,** Amin N, Marino M, Gotfried M, Meyer T, Sommerer K, Baughman RA. Insulin lung deposition and clearance following Technosphere® insulin inhalation powder administration. *Pharm Res* 2011; **28**: 2157-2164 [PMID: 21491144 DOI: 10.1007/s11095-011-0443-4]
- 4 **Kugler AJ,** Fabbio KL, Pham DQ, Nadeau DA. Inhaled technosphere insulin: a novel delivery system and formulation for the treatment of types 1 and 2 diabetes mellitus. *Pharmacotherapy* 2015; **35**: 298-314 [PMID: 25809179 DOI: 10.1002/phar.1555]
- 5 **Potocka E,** Cassidy JP, Haworth P, Heuman D, van Marle S, Baughman RA. Pharmacokinetic characterization of the novel pulmonary delivery excipient fumaryl diketopiperazine. *J Diabetes Sci Technol* 2010; **4**: 1164-1173 [PMID: 20920436 DOI: 10.1177/193229681000400515]
- 6 **Heinemann L,** Baughman R, Boss A, Hompesch M. Pharmacokinetic and Pharmacodynamic Properties of a Novel Inhaled Insulin. *J Diabetes Sci Technol* 2016; pii: 1932296816658055 [PMID: 27378794 DOI: 10.1177/1932296816658055]
- 7 **Afrezza.** Prescribing Information. MannKind Corporation. Danbury, CT, June 2014
- 8 **Rave K,** Potocka E, Heinemann L, Heise T, Boss AH, Marino M, Costello D, Chen R. Pharmacokinetics and linear exposure of AFRESA compared with the subcutaneous injection of regular human insulin. *Diabetes Obes Metab* 2009; **11**: 715-720 [PMID: 19476477 DOI: 10.1111/j.1463-1326.2009.01039.x]
- 9 **Rosenstock J,** Franco D, Korpachev V, Shumel B, Ma Y, Baughman R, Amin N, McGill JB. Inhaled Technosphere Insulin Versus Inhaled Technosphere Placebo in Insulin-Naïve Subjects With Type 2 Diabetes Inadequately Controlled on Oral Antidiabetes Agents. *Diabetes Care* 2015; **38**: 2274-2281 [PMID: 26253730 DOI: 10.2337/dc15-0629]
- 10 **Bode BW,** McGill JB, Lorber DL, Gross JL, Chang PC, Bregman DB. Inhaled Technosphere Insulin Compared With Injected Prandial Insulin in Type 1 Diabetes: A Randomized 24-Week Trial. *Diabetes Care* 2015; **38**: 2266-2273 [PMID: 26180109 DOI: 10.2337/dc15-0075]
- 11 **Rosenstock J,** Bergenstal R, Defronzo RA, Hirsch IB, Klonoff D, Boss AH, Kramer D, Petrucci R, Yu W, Levy B. Efficacy and safety of Technosphere inhaled insulin compared with Technosphere powder placebo in insulin-naïve type 2 diabetes suboptimally controlled with oral agents. *Diabetes Care* 2008; **31**: 2177-2182 [PMID: 18678610 DOI: 10.2337/dc08-0315]
- 12 **Rosenstock J,** Lorber DL, Gnudi L, Howard CP, Bilheimer DW, Chang PC, Petrucci RE, Boss AH, Richardson PC. Prandial inhaled insulin plus basal insulin glargine versus twice daily biphasic insulin for type 2 diabetes: a multicentre randomised trial. *Lancet* 2010; **375**: 2244-2253 [PMID: 20609970 DOI: 10.1016/S0140-6736(10)60632-0]
- 13 **Pittas AG,** Westcott GP, Balk EM. Efficacy, safety, and patient acceptability of Technosphere inhaled insulin for people with diabetes: a systematic review and meta-analysis. *Lancet Diabetes Endocrinol* 2015; **3**: 886-894 [PMID: 26341170 DOI: 10.1016/S2213-8587(15)00280-6]
- 14 **Raskin P,** Heller S, Honka M, Chang PC, Boss AH, Richardson PC, Amin N. Pulmonary function over 2 years in diabetic patients treated with prandial inhaled Technosphere Insulin or usual antidiabetes treatment: a randomized trial. *Diabetes Obes Metab* 2012; **14**: 163-173 [PMID: 21951325 DOI: 10.1111/j.1463-1326.2011.01500.x]
- 15 **Lasagna-Reeves CA,** Clos AL, Midoro-Hiriuti T, Goldblum RM, Jackson GR, Kaye R. Inhaled insulin forms toxic pulmonary amyloid aggregates. *Endocrinology* 2010; **151**: 4717-4724 [PMID: 20685871 DOI: 10.1210/en.2010-0457]
- 16 **Bloomgarden ZT.** Afrezza: some questions about a new approach to prandial insulin. *J Diabetes* 2014; **6**: 489-490 [PMID: 25209874 DOI: 10.1111/1753-0407.12217]
- 17 **Angelo R,** Rousseau K, Grant M, Leone-Bay A, Richardson P. Technosphere insulin: defining the role of Technosphere particles at the cellular level. *J Diabetes Sci Technol* 2009; **3**: 545-554 [PMID: 20144294 DOI: 10.1177/193229680900300320]
- 18 **Mayer P,** Reitzenstein U, Warnken M, Enzmann H, Racké K. Insulin action on H292 bronchial carcinoma cells as compared to normal bronchial epithelial cells. *Pulm Pharmacol Ther* 2012; **25**: 104-114 [PMID: 22210006 DOI: 10.1016/j.pupt.2011.12.005]
- 19 **Potocka E,** Amin N, Cassidy J, Schwartz SL, Gray M, Richardson PC, Baughman RA. Insulin pharmacokinetics following dosing with Technosphere insulin in subjects with chronic obstructive pulmonary disease. *Curr Med Res Opin* 2010; **26**: 2347-2353 [PMID: 20804443 DOI: 10.1185/03007995.2010.511971]
- 20 **Zisser H,** Dassau E, Lee JJ, Harvey RA, Bevier W, Doyle FJ. Clinical results of an automated artificial pancreas using technosphere inhaled insulin to mimic first-phase insulin secretion. *J Diabetes Sci Technol* 2015; **9**: 564-572 [PMID: 25901023 DOI: 10.1177/1932296815582061]
- 21 **Goldberg T,** Wong E. Afrezza (Insulin Human) Inhalation Powder: A New Inhaled Insulin for the Management Of Type-1 or Type-2 Diabetes Mellitus. *P T* 2015; **40**: 735-741 [PMID: 26609206]

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Basic Study

## Evaluation of extraction protocols for anti-diabetic phytochemical substances from medicinal plants

Stanley Irobehkian Reuben Okoduwa, Ismaila A Umar, Dorcas B James, Hajara M Inuwa, James D Habila

Stanley Irobehkian Reuben Okoduwa, Directorate of Research and Development, Nigerian Institute of Leather and Science Technology, Zaria 810004, Nigeria

Stanley Irobehkian Reuben Okoduwa, Ismaila A Umar, Dorcas B James, Hajara M Inuwa, Department of Biochemistry, Ahmadu Bello University, Zaria 811104, Nigeria

James D Habila, Department of Chemistry, Ahmadu Bello University, Zaria 811104, Nigeria

**Author contributions:** Okoduwa SIR and Umar IA got the concept and design of the study; Okoduwa SIR and Habila JD perform the study and data analysis; James DB, Inuwa HM and Habila JD participated in the interpretation of data; Okoduwa SIR wrote the first version of the manuscript; Umar IA, James DB, Inuwa HM and Habila JD critically revised the manuscript for important intellectual content; all the authors read and gave a final approval of the revised version of the manuscript to be published.

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**Institutional animal care and use committee statement:** The experimental protocol was review and approved by the Research Ethics Committee of the Institute. All experimental protocol was in conformity with the Institutional guidelines that are in compliance with National and International Laws and Guidelines for Care and Use of Laboratory Animals in Biomedical Research.

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**Correspondence to:** Stanley Irobehkian Reuben Okoduwa, Researcher, Directorate of Research and Development, Nigerian Institute of Leather and Science Technology, P.M.B. 1034, Zaria 810004, Nigeria. [siroplc@gmail.com](mailto:siroplc@gmail.com)  
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## Abstract

### AIM

To examine the efficacy of three extraction techniques: Soxhlet-extraction (SE), cold-maceration (CM) and microwave-assisted-extraction (MAE) using 80% methanol as solvent.

### METHODS

The study was performed on each of 50 g of *Vernonia amygdalina* (VA) and *Occimum gratissimum* (OG) leaves respectively. The percentage yield, duration of extraction, volume of solvent used, qualitative and quantitative phytoconstituents present was compared. The biological activities (hypoglycemic effect) were investigated using albino wistar rat model of diabetes mellitus ( $n = 36$ ) with a combined dose (1:1) of the two plants leaf extracts (250 mg/kg b.w.) from the three methods. The

extracts were administered orally, once daily for 21 d.

## RESULTS

In this report, the percentage VA extract yield from MAE was highest ( $20.9\% \pm 1.05\%$ ) within 39 min using 250 mL of solvent, when compared to the CM ( $14.35\% \pm 0.28\%$ ) within 4320 min using 900 mL of solvent and SE ( $15.75\% \pm 0.71\%$ ) within 265 min using 500 mL of solvent. The percentage differences in OG extract yield between: MAE *vs* SE was 41.05%; MAE *vs* CM was 46.81% and SE *vs* CM was 9.77%. The qualitative chemical analysis of the two plants showed no difference in the various phytoconstituents tested, but differs quantitatively in the amount of the individual phytoconstituents, as MAE had significantly high yield ( $P > 0.05$ ) on phenolics, saponins and tannins. SE technique gave significantly high yield ( $P > 0.05$ ) on alkaloid, while CM gave significant high yield on flavonoids. The extracts from CM exhibited a significantly ( $P > 0.05$ ) better hypoglycemic activity within the first 14-d of treatment ( $43.3\% \pm 3.62\%$ ) when compared to MAE ( $36.5\% \pm 0.08\%$ ) and SE methods ( $33.3\% \pm 1.60\%$ ). However, the percentage hypoglycemic activity, 21 d post-treatment with 250 mg/kg b.w. extract from MAE was  $72.6\% \pm 1.03\%$  and it was more comparable to 10 mg/kg b.w. glibenclamide treated group ( $75.0\% \pm 0.73\%$ ), unlike the SE ( $69.5\% \pm 0.71\%$ ) and CM ( $69.1\% \pm 1.03\%$ ).

## CONCLUSION

CM technique produces extract with better hypoglycemic activity, whereas; MAE is a better option for high yield of phytoconstituents using less solvent within a short time.

**Key words:** Extraction techniques; Microwave-assisted-extraction; Maceration; Phytoconstituents; Medicinal plants; Soxhlet; Anti-diabetes

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**Core tip:** Extraction of active phytoconstituents from medicinal plants rely mostly on the use of appropriate extraction method. Different extraction techniques affect the yield and biological activity of phytocomponents. In this study, we observed that microwave assisted extraction produces significantly higher overall extract yield as well as in phenolic, saponin and tannin content. Cold maceration and soxhlet extraction produced higher flavonoid and alkaloid yield respectively. Maceration extracts exhibited significantly better hypoglycemic activities in diabetic rats compared to extracts from soxhlet and microwave assisted extraction. This study reveals that the choice of extraction protocol should depend primarily on the purpose of interest.

Okoduwa SIR, Umar IA, James DB, Inuwa HM, Habila JD. Evaluation of extraction protocols for anti-diabetic phytochemical substances from medicinal plants. *World J Diabetes* 2016; 7(20):

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## INTRODUCTION

Diabetes mellitus (DM) is one of the most common non-communicable diseases globally, affecting the quality of human life of all ages across the world<sup>[1,2]</sup>. The disease has become a global public health problem affecting the socio-economic status of the individual<sup>[3]</sup>. It is an age long, serious heterogeneous metabolic disorder characterized by hyperglycemia and glucose intolerance, due to endogenous insulin deficiency, impaired effectiveness of insulin action, or both<sup>[4]</sup>. With DM the body cannot regulate the amount of sugar in the blood. This leads to increased glucose in the body that causes deregulation of the metabolism, often accompanied by glycosuria, polydipsia, and polyuria<sup>[4]</sup>.

According to International Diabetic Foundation report, every 6 s, a person dies from diabetes<sup>[5]</sup>. In 2013, 5.0 million deaths were recorded across the globe with a prevalence of 8.3%<sup>[5]</sup>. A total of 415 million people are affected with diabetes, worldwide as at December, 2015. The figure is estimated to rise above 642 million by 2040<sup>[5]</sup>. In Nigeria, 3921500 cases have been reported as at 2013 with a prevalence rate of 4.99%. This alarming rate calls for urgency to find better treatment and novel prevention strategies for the disease.

Several hypoglycemic drugs are available for managing diabetes since it is incurable but they suffer from generally inadequate efficacy and number of serious adverse effects<sup>[6,7]</sup>. Hence, the shift to the use of plant source, a new hopeful approach that has long been authenticated by World Health Organization in its general assembly<sup>[8]</sup>.

Plants are the major source of potential therapeutic agents worldwide. The uses of Plants for therapeutic purposes have been recorded to be as long as history. Plants which contain substances that could be used for medicinal purposes or which are precursors for the synthesis of useful drugs are considered as therapeutic plants<sup>[9]</sup>. According to the report of Farnsworth and Soejarto<sup>[10]</sup>, there are between 35000 and 70000 plant species that have been used for medicinal purposes in the world<sup>[10]</sup>. The bioactive components present in plants can only be utilized in disease treatment/management after being extracted with suitable solvent and prepared into substances such as ointment, cream, gel, moisturizer, pills and so on<sup>[11,12]</sup>.

Extraction is the partitioning of therapeutically dynamic segments of plant utilizing suitably selective solvents through standard methods<sup>[12,13]</sup>. The basis of all extraction in medicinal plant research is to isolate the dissolvable plant metabolites, excluding the insoluble cell marc. The preliminary unrefined extracts obtained utilizing these strategies contain complex blend of numerous plant metabolites, for example, alkaloids,

glycosides, phenolics, terpenoids and flavonoids<sup>[12,14,15]</sup>. Some bioactive components present in plants are either heat sensitive or solvent specific hence the quality and composition of the extracts as well as their biological activities are affected by the type of extraction procedure. To get the most astounding biological efficacy and yield of plant extract, it is important to consider these limitations and utilize a standardized method for a specific bioactive molecule<sup>[14,16]</sup>.

The needs of standardized extraction methodology for unrefined medications are to accomplish the remedially craved part and to dispose of the inactive portion by treatment with a specific solvent called menstruum. The extract consequently obtained might be prepared for use as a therapeutic agent in the form of tinctures and fluid extracts, it may be further processed to be fused in any dosage form such as tablets or capsules, or it might be fractionated to seclude singular synthetic substances, for example, vincristine, hyoscyne and ajmalicine which are modern drugs. For that reason, standardization of extraction methodologies contributes fundamentally to the final nature of the medicinal drug<sup>[13,15]</sup>.

However, with the expanding interest for natural therapeutic products and nutraceuticals for healthcare everywhere throughout the world, producers of medicinal plant extracts are in continuous search for the most suitable extraction strategy keeping in mind the end goal to produce extracts of characterized quality with minimal variability from batch to batch. Conventional extraction is generally carried-out using reflux, maceration, soxhlet and distillation techniques. These techniques which have been utilized for a long time are extremely tedious and require generally a lot of solvents. Extraction utilizing non-routine techniques, for instance, microwave techniques can produce high yield, within a shorter time utilizing a smaller amount of solvents<sup>[17,18]</sup>. Among the different customary and routine extraction systems, Soxhlet extraction has been the most generally utilized. Unfortunately, there is paucity of literature on the best extraction method for a specific bioactive molecule. The evaluation on some of these methods by most previous investigators focuses on either the yield or duration of extraction, without considering the effects of the protocol on the various bioactive entities that works in a synergic manner.

Soxhlet extraction serves not just as a method for extraction of phyto-constituents but additionally as a reference to look at more current extraction procedures. Previously, it has been suggested that the microwave-assisted-extraction (MAE), a present day extraction method is a superior method for extricating phyto-components from plants<sup>[19]</sup>. Several reports on the usefulness of the MAE as it concerns medicinal plants have been published<sup>[20-24]</sup>.

*Vernonia amygdalina* (VA) commonly known as bitter leaf and *Occimum gratissimum* (OG) generally refers to as scent leaf, have been reported to have anti-diabetic properties<sup>[25]</sup>. The efficacy of the combined use of both

plants with respect to diabetes has been documented<sup>[26]</sup>. Also, their hypoglycemic activities have been attributed to the presence of flavonoids, alkaloids and saponins among others<sup>[27-29]</sup>. It is therefore imperative to examine the effect of different extraction methods on their biological activities.

From our insight, the extraction of phytoconstituents from VA and OG using MAE strategy has not yet been accounted for. These two plants were chosen as a reference point for other therapeutic plants owing to the fact that their anti-diabetic potentials have been established in recent publications<sup>[26-31]</sup>. Therefore, this study evaluated three extraction technologies, viz: MAE, Soxhlet extraction and cold maceration with an aim to present a comparison among the distinctive strategies utilized for extraction of hypoglycemic bioactive constituents from medicinal plants. The primary goal of the research is to give a successful and effective, straightforward, safe and less time consuming with maximal yield strategy for extricating specific bioactive parts from therapeutic plants.

## MATERIALS AND METHODS

### Plant preparation

Fresh specimen of VA and OG leaves were harvested in the month of May, 2015 from a local farm in Samaru, Zaria, Kaduna State, Nigeria. The plant samples were identified and authenticated by the Herbarium unit of the Department of Biological Science, Ahmadu Bello University, Zaria, Nigeria. A voucher specimen number 1166 and 1285 were deposited for VA and OG respectively. The leaves of the plants sample were dried under shade at room temperature to constant weights for seven days. The dried samples were then pulverized into powder using a laboratory milling machine (Thomas-Wiley Laboratory mill Model 4, United States). The powders were preserved in clean plastic containers, kept away from light, heat and moisture until use.

### Reagents and chemicals

All the chemicals and reagents used were of analytical reagent grade.

### Experimental duration

The research was conducted between May and December, 2015.

### Apparatus

A conventional microwave oven (2450 MHz, Toshiba, and Tokyo, Japan) with variable power up to 1000 watts, a time controller, beam reflector and a stirring device was used.

### Procedure

Two conventional extraction techniques namely, Soxhlet and cold maceration were used in comparison with a new modern technology, the MAE technique.



### Soxhlet extraction method

Exhaustive Soxhlet extraction was performed using classical apparatus with accurately weighed 50 g of the powdered leaf samples of VA and OG respectively. Extraction was performed with 80% methanol as the extraction solvent. After extraction, the methanol solvent was evaporated by concentrating under vacuum with rotary evaporator (Senco Rotary Evaporator, Model RE 801) at 40 °C under reduced pressure. The solvent free methanol extract was thereafter evaluated.

### Cold maceration method

Maceration was carried out in a closed conical flask for 72 h. In both case 50 g powdered VA and OG leaf sample and 80% methanol as the extraction solvent were used. The suspension after maceration was centrifuged and the supernatant evaporated under reduce pressure. The solvent free methanol extracts obtained were similarly evaluated.

### Microwave assisted extraction method

Accurately weighed 50 g of the homogeneous powder leaf samples was mixed with 60 mL, 80% methanol. After allowing a preleaching time of 5 min the suspension was irradiated with microwave at optimized conditions<sup>[32-34]</sup>. The samples were treated under microwave irradiation in an intermittent way, *i.e.*, Irradiation: cooling: irradiation. The microwave irradiation time was set at three minutes and cooling time of five minutes was allowed. After 5 repeats, the samples were centrifuged at 4000 rpm and the supernatant evaporated under pressure. The dried residue was evaluated accordingly.

### Percentage recovery yield of extraction

The percentage extraction yield (w/w) by the three extraction methods was calculated using the formula: Percentage extraction yield for plant extract = [mass of extract (g)/mass of plant sample (g)] × 100

Aliquots of the extracts were stored in screwed cap vials at 4 °C-8 °C until further use. The extracts were re-dissolved in distilled water when required and given orally through gastric intubations.

### Phytochemical analysis

Standard protocols were used in detecting the phytochemical constituents present in the two plants samples<sup>[35,36]</sup>. Tannins according the method describe by Markkar *et al*<sup>[37]</sup>, Saponins as described by Bruneton<sup>[38]</sup>, Alkaloids as described by Harbone<sup>[39]</sup>, Flavonoids as described by Bohm *et al*<sup>[40]</sup>.

### Biological assay

To test biological activities of the plant extract from the different extraction techniques, thirty six albino wistar rats (150-200 g of either sex) fed with rat pellet diet (Grand Cereals Ltd, Nigeria) and water *ad libitum* were used. Animals were first acclimatized for two weeks before used. The study was conducted at the Research

and Development Laboratory of Nigerian Institute of Leather and Science Technology (NILEST), Zaria Nigeria. The anti-hyperglycemic effect of the extracts obtained from the three extraction methods were assessed using rat model of DM. The experimental protocol was approved by the Institutional Animal Ethic Committee. All experimental protocol was in conformity with the institutional guidelines that are in compliance with National and International Laws and Guidelines for Care and Use of Laboratory Animals in Biomedical Research. The rules and regulations in accordance to the Ethical Committee directive were strictly followed.

### Induction of diabetes

The rat model of diabetes used for this study was developed as followed. First, the rats were fasted overnight after which they were given a single intra-peritoneal injection (*ip*) of 55 mg/kg b.w. of streptozotocin (STZ) (Adooq Bioscience, LLC, United States) dissolved in 0.1 mL fresh cold citrate buffer pH 4.5.

Confirmation of diabetes was done 72 h after STZ induction, using a One Touch Glucometer (Lifescan Inc 1995 Milpas, California, United States). Blood samples were obtained from the tail puncture of the rats. Animals with fasting blood glucose  $\geq 200$  mg/dL, after 10 d of STZ induction were considered diabetic and included in the study as diabetic animals<sup>[30]</sup>.

### Experimental design

Thirty six rats were divided into 6 groups of 6 rats per group. The treatments were as follows: (1) diabetic rats treated with extracts from cold maceration 250 mg/kg b.w.; (2) diabetic rats treated with extracts from soxhlet extraction (SEE) 250 mg/kg b.w.; (3) diabetic rats treated with extracts from MAE 250 mg/kg; (4) diabetic rats as positive control treated with standard drug (10 mg/kg b.w, glibenclamide); (5) diabetic rats as negative control. No treatment was given; and (6) non-diabetic rats as standard control (no induction, no treatment).

### Route of administration

The extracts were administered orally, once daily for 21 d using combined dose (1:1) of the two plants leaf extracts (250 mg/kg b.w.)

### Blood sample collection

Blood sample was withdrawn from the tail vein and tested using glucose test strips and glucometer (On-Call Plus, Acon Laboratories Ins, United States) after an overnight fast.

### Statistical analysis

The results obtained were expressed as mean  $\pm$  SD where applicable. The data were analyzed using analysis of variance and significant differences among means were determined by Duncan's multiple range test at  $P < 0.05$  using Statistical Package for Social Sciences software version 20 for windows.

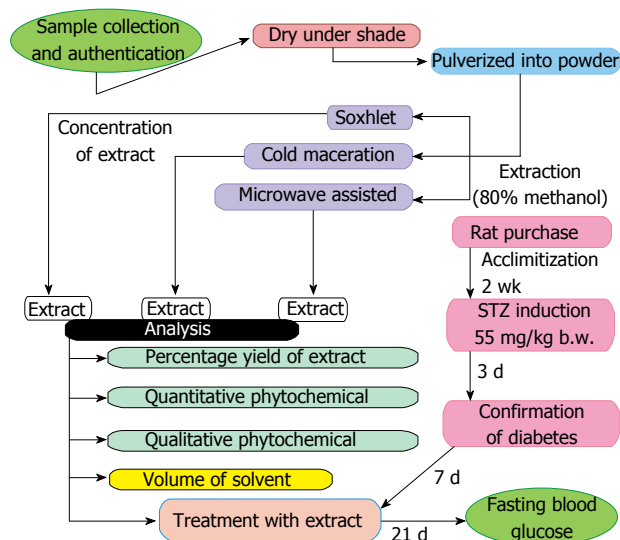


Figure 1 Experimental design for the evaluation of three extraction methods. STZ: Streptozotocin.

## RESULTS

The efficacy of three extraction methods were compared by evaluating the anti-hyperglycemic effects of the two medicinal plants (VA and OG) leaf extracts. A flow chart illustrating the experimental design in detail is presented in Figure 1. The experiments were performed using the same quantity of plant samples (50 g each) and the biological activities were analyzed using rat model of diabetes with the same dose of extract (250 mg/kg body weight). A comparison of the extraction of the medicinal plants (VA and OG) using conventional microwaves, soxhlet extractor and cold maceration are shown in Table 1.

The yield of MAE extracts from VA for 39 min were higher ( $20.90\% \pm 1.05\%$ ) than that of soxhlet extract ( $15.75\% \pm 0.71\%$ ) for 265 min and that of maceration extract ( $14.35\% \pm 0.28\%$ ) for 4320 min. Similarly, the yield of microwave extracts from OG ( $19.10\% \pm 1.67\%$ ) for 39 min were higher than that of soxhlet extract ( $11.25\% \pm 0.42\%$ ) for 255 min and that of maceration extract ( $10.15\% \pm 0.65\%$ ) for 4320 min. Regarding the extraction time, our result show that MAE appeared to be the fastest method since the extraction could be achieved within minutes. The MAE also consumed the least amount of solvent 250 mL when compared to the other methods, 500 mL and 900 mL for soxhlet and cold maceration respectively (Table 1).

The percentage differences in the recovery yield between two specific extraction methods are shown in the Table 2. It was observed that for VA, the difference in percentage recovery yield between MAE vs soxhlet extraction method was 24.59%; but with OG it was 41.05%. The difference in percentage yield between MAE vs cold maceration method was 31.29% for VA and 46.81% for OG. Although there were no significant differences between the soxhlet and cold maceration method for both plants, the percentage difference was

8.88% and 9.77 for VA and OG respectively (Table 2).

The results from the present study showed that there was no difference between the qualitative phytoconstituents obtained by the various extraction technologies under investigation (Table 3).

However, the quantitative chemical analysis (Table 4) revealed a statistically higher yield in alkaloid from the soxhlet extraction when compared to the MAE and cold maceration for VA. But there was no significant difference in the alkaloid yield between the soxhlet and MAE method for OG. The cold maceration technology recorded the highest yield in flavonoid in both plant when compared to the MAE and soxhlet extraction method. The difference was statistically significant ( $P < 0.05$ ). The MAE had the highest yield in phenolics, saponins and tannins from the two plants studied when compared to the conventional extraction techniques (soxhlet and cold maceration). However, there was no significant difference between MAE and soxhlet in the yield of tannin from VA.

Soxhlet, cold maceration and MAE extracts of VA and OG were tested and compared for anti-diabetic activities. All the extracts exhibited comparable anti-diabetic activities with that of standard drug (glibenclamide) under the same dose rate of 250 mg/kg body weight tested according to the method of Abdulazeez *et al.*<sup>[26]</sup>. The cold maceration extract exhibited a better hypoglycemic effect within the first 7 (17.9%) and 14 (43.2%) d of treatment when compared to others. But the hypoglycemic activity of extract obtained from MAE was more comparable to the standard drug glibenclamide in reducing the blood glucose of the animals after 28 d post induction (21 d treatment).

The extract from MAE exhibited the least hypoglycemic effect within the first 7 day of treatment. There was no significant difference between the hypoglycemic effects of extracts obtained from soxhlet and the cold maceration method at the end of the 21 d (Tables 5 and 6).

## DISCUSSION

Developing nations are rich in therapeutic plants at the same time, because of trouble in getting reliably effective extraction equipments; esteem expansion to this rich bioresources are difficult. Usually and prevalently in extremely poor nations, the advancements utilized are improper and not efficient. The crucial setback is identified with the nature of the products. Primitive extraction techniques don't promise a steady and top notch reliable quality and, sometimes, unseemly innovations and techniques result in creating defiled products which has low market value<sup>[41]</sup>. The present study, evaluated three different technologies for extraction of hypoglycemic compounds from medicinal plants, with a view to ascertain the best option for the isolation of specific bioactive entity.

In the study it was noticed that Soxhlet extraction and cold maceration spent longer time for complete extraction of same quantity of the plant sample studied. Also, the amount of the solvent utilized in MAE was

**Table 1** Comparison of percentage recovery yield, extraction time and volume of solvent used for the different extraction methods for the two plants

Parameter	<i>Vernonia amygdalina</i>			<i>Ocimum gratissimum</i>		
	MAE	Soxhlet	Maceration	MAE	Soxhlet	Maceration
Sample (g)	50	50	50	50	50	50
Solvent volume (mL)	250	500	900	250	500	900
Extraction time (min)	39	265	4320	39	255	4320
Recovery yield (g)	10.45 ± 0.53 <sup>b</sup>	7.88 ± 0.35 <sup>a</sup>	7.18 ± 0.14 <sup>a</sup>	9.55 ± 0.84 <sup>b</sup>	5.63 ± 0.21 <sup>a</sup>	5.08 ± 0.32 <sup>a</sup>
Percentage recovered (%)	20.90 ± 1.05 <sup>b</sup>	15.75 ± 0.71 <sup>a</sup>	14.35 ± 0.28 <sup>a</sup>	19.10 ± 1.67 <sup>b</sup>	11.25 ± 0.24 <sup>a</sup>	10.15 ± 0.65 <sup>a</sup>

Values are mean ± SD of 3 replicate determinations; same superscript across the column under the same plant indicate no significant difference ( $P > 0.05$ ). MAE: Microwave-assisted-extraction.

**Table 2** Calculated percentage differences in recovery yield of extracts between the extraction methods

<i>Vernonia amygdalina</i>	<i>Ocimum gratissimum</i>		
	MAE	Soxhlet	Maceration
MAE		41.05%	46.81%
Soxhlet	24.59%		9.77%
Maceration	31.29%	8.88%	

Percentage difference between two extraction methods = [(values from method with higher recovery yield - values from method with lower recovery yield)/values from method with higher recovery yield] × 100.

less, proving that, MAE is indeed truly economical. Comparable results were reported by Vongsangnak *et al.*<sup>[42]</sup>, when contrasting routine extraction procedures with MAE throughout the isolation of saponins from cell culture of *Panax notoginseng* and analgesic substances from the roots of *Ximenia americana*<sup>[20]</sup>. Besides, microwave illumination technique has been reported to be extremely quick, dependable for making of Schiff bases<sup>[21]</sup>. Possible explanation observed in the various methods of extraction could be due to differences in rate of chemical reaction. For instance, in soxhlet extraction approach, the applied heat supplies the activation energy needed to rupture the plant tissues in order to release its phytoconstituents. Whereas in MAE, the activation energy is achieved due to oscillatory wave generated by the system and by the ionic conduction and dipole rotation of the molecules of the plant sample. This brings about increase in the cell pressure, thereby rupturing to release its contents on attaining its elastic limit. The heat in MAE is internally generated by the molecules unlike in soxhlet whereby the heat is externally generated. In cold maceration, the release of phytoconstituents from plant matrix is due to differences in ionic solvent concentration gradients, hence more solvent is required to create a positively dynamic concentration gradients. This accounts for the higher volume of solvent utilized by the cold maceration as observed in the present study. In a nutshell, with MAE, the plant cell tissues are effectively broken and separated to release phytoconstituents with ease, hence the greater yield as recorded.

The result of the quantitative phytochemical screening within the limit of the analyzed components in the present study suggests that the phytoconstituents of the

**Table 3** Comparison of some qualitative phytochemicals obtained with the three extraction methods for the two plants

S/N	Constituents	Maceration		Soxhlet		Microwave	
		VA	OG	VA	OG	VA	OG
1	Carbohydrates	+	+	+	+	+	+
2	Anthraquinones	-	-	-	-	-	-
3	Glycosides	+	+	+	+	+	+
4	Cardiac glycosides	+	+	+	+	+	+
5	Saponins	+	+	+	+	+	+
6	Steroids	+	+	+	+	+	+
7	Triterpenes	+	+	+	+	+	+
8	Tannins	+	+	+	+	+	+
9	Flavonoids	+	+	+	+	+	+
10	Alkaloid	+	+	+	+	+	+

The symbol (+): Indicate detected; (-): Not detected; VA: *Vernonia amygdalina*; OG: *Ocimum gratissimum*.

studied plants were not destroyed since the results obtained showed that the same phytoconstituents tested in all the extracts from the different procedures were the same. This finding is in agreement with earlier reports of Mandal *et al.*<sup>[19]</sup>, that, the plant components obtained from MAE are neither decomposed nor oxidized under optimized conditions. Several phytochemicals have been found to give an increase in their extractive yields when compared to their yields on subjection to conventional extraction techniques<sup>[21]</sup>. Chan *et al.*<sup>[22]</sup> also recorded a higher yield in the extraction of anti-diabetic ingredient from herbal plant. In their experiment using 5 g sample with 150 mL solvent recorded a yield of 1.63 mg/g sample in 5 min using MAE as against 0.47 mg/g in 3 h obtained with soxhlet extraction technology. In a related study for extraction of caffeine and polyphenols from leaves of green tea, MAE achieved higher extraction yield within 4 min than any extraction methods at room temperature for 20 h<sup>[43]</sup>. Ginsenosides extraction yield from ginseng root was obtained in 15 min using focused MAE technique which was also better than other conventional solvent extraction technologies for 10 h<sup>[24,44]</sup>. The higher yield obtained from MAE and soxhlet extraction when compared to the cold maceration may be attributed to the heat exchange and mass transfer.

In soxhlet extraction, the heat exchange and the mass transfer are restricting variables contrast with MAE, where heat exchange happens from the focal point of

**Table 4** Comparison of the quantitative phytochemicals obtained with the three extraction methods for the two plants

	<i>Vernonia amygdalina</i> (mg/100 g)			<i>Ocimum gratissimum</i> (mg/100 g)		
	MAE	Soxhlet	Maceration	MAE	Soxhlet	Maceration
Alkaloid	4.0 ± 0.65 <sup>a</sup>	7.0 ± 0.57 <sup>b</sup>	5.0 ± 0.46 <sup>a</sup>	6.5 ± 0.75 <sup>b</sup>	7.0 ± 0.15 <sup>b</sup>	5.0 ± 0.90 <sup>a</sup>
Flavonoid	13.0 ± 0.35 <sup>b</sup>	9.0 ± 0.50 <sup>a</sup>	15.0 ± 0.21 <sup>c</sup>	10.0 ± 0.35 <sup>b</sup>	8.0 ± 0.18 <sup>a</sup>	12.0 ± 0.25 <sup>c</sup>
Phenolic	17.5 ± 0.25 <sup>c</sup>	15.0 ± 0.21 <sup>b</sup>	12.0 ± 0.70 <sup>a</sup>	15.0 ± 0.22 <sup>c</sup>	14.0 ± 0.21 <sup>b</sup>	11.0 ± 0.65 <sup>a</sup>
Saponin	4.5 ± 0.45 <sup>b</sup>	3.0 ± 0.30 <sup>a</sup>	2.0 ± 0.75 <sup>a</sup>	6.1 ± 0.75 <sup>b</sup>	4.0 ± 0.92 <sup>a</sup>	5.0 ± 0.20 <sup>a</sup>
Tannin	14.0 ± 0.55 <sup>b</sup>	13.0 ± 0.50 <sup>b</sup>	10.0 ± 0.25 <sup>a</sup>	12.0 ± 0.18 <sup>b</sup>	9.0 ± 0.24 <sup>a</sup>	9.1 ± 0.25 <sup>a</sup>

Values are mean ± SD of 3 replicate readings. Same superscript across the column under the same plant signifies no significant difference at  $P > 0.05$ . MAE: Microwave-assisted-extraction.

**Table 5** Effect of the extract from the different extraction methods on blood glucose in streptozotocin-induced diabetic rats

Group	Before induction	7 d after induction	Days after treatment		
			7 <sup>th</sup>	14 <sup>th</sup>	21 <sup>st</sup>
CME	79.4 ± 4.5	344.6 ± 10.3	282.8 ± 17.6	195.8 ± 18.3	106.5 ± 6.7
SEE	85.3 ± 3.2	396.8 ± 15.7	342.5 ± 14.7	264.9 ± 16.8	121.1 ± 10.2
MAE	82.7 ± 5.1	373.3 ± 13.6	331.3 ± 11.4	237.1 ± 8.9	102.4 ± 7.6
PC	91.6 ± 4.5	389.4 ± 11.9	359.0 ± 12.6	266.3 ± 13.2	97.3 ± 5.8
NC	80.6 ± 3.8	365.5 ± 9.8	395.1 ± 16.3	419.6 ± 10.2	448.4 ± 17.4
HC	83.2 ± 6.2	84.8 ± 4.7	83.9 ± 7.8	84.7 ± 6.5	84.2 ± 7.1

Values are mean ± SD (mg/dL) of readings from 6 rats per group. CME: Cold maceration extract; SEE: Soxhlet extraction extract; MAE: Microwave-assisted extraction extract; PC: Positive control; NC: Negative control; HC: Healthy control.

**Table 6** Calculated percentage change in blood glucose after treatment with the extracts in streptozotocin-induced diabetic rats

Group	Initial blood glucose on day 0 (mg/dL)	Percentage change after treatment (%)		
		Day 7	Day 14	Day 21
CME	344.6 ± 10.3	18.0 ± 2.66 <sup>c</sup>	43.3 ± 3.62 <sup>c</sup>	69.1 ± 1.03 <sup>c</sup>
SEE	396.8 ± 15.7	13.7 ± 0.29 <sup>d</sup>	33.3 ± 1.60 <sup>c,d</sup>	69.5 ± 0.71 <sup>c</sup>
MAE	373.3 ± 13.6	11.2 ± 0.18 <sup>c,d</sup>	36.5 ± 0.08 <sup>d</sup>	72.6 ± 1.03 <sup>d</sup>
PC	389.4 ± 11.9	7.8 ± 0.42 <sup>c</sup>	31.6 ± 1.30 <sup>c</sup>	75.0 ± 0.73 <sup>d</sup>
NC	365.5 ± 9.8	-8.1 ± 1.57 <sup>a</sup>	-14.8 ± 0.29 <sup>a</sup>	-22.7 ± 1.47 <sup>a</sup>
HC	84.8 ± 4.7	1.4 ± 3.96 <sup>b</sup>	0.1 ± 2.12 <sup>b</sup>	0.7 ± 2.88 <sup>b</sup>

Same superscript down the column signifies no significant difference at  $P > 0.05$ . Percentage change in blood glucose = [(blood glucose before treatment - blood glucose after treatment)/blood glucose before treatment] × 100. CME: Cold maceration extract; SEE: Soxhlet extraction extract; MAE: Microwave-Assisted Extraction extract; PC: Positive control; NC: Negative control; HC: Healthy control.

the specimens to the external colder environment, and volumetric warming impact prompts a speedier ascend in temperature. Additionally, the interior warming of *in situ* water inside of the plant material expands the plant cells and prompts the burst of the plant tissues<sup>[45]</sup>. This may be the explanation behind the higher extraction of phytochemicals from MAE when compared to soxhlet and cold maceration extracts observed in the present study. It is very obvious from these results, that microwave extraction represents a promising substitute for extracting hypoglycemic compounds from natural substrate.

The hypoglycemic activity of the 250 mg/kg b.w. cold

maceration extract was significantly better than others within the first 14 day of treatment. Surprisingly after 21 d treatment, the blood glucose lowering capacity of the MAE extract was almost the same with that of 10 mg/kg b.w. glibenclamide. The percentage change in blood glucose by MAE extract after the experimental duration of 21 d treatment was 72.6% whereas that obtained from the standard drug glibenclamide was 75.0%. The higher yield of alkaloid by soxhlet extraction may account for its better efficacy when compared to the cold maceration in the present study. Saponin, alkaloid and flavonoid are known to play a significant role in anti-diabetic action<sup>[27-29]</sup>.

MAE had the highest yield in three different phyto-constituents *viz* phenolics, saponins and tannins. This suggests that microwave methanol extract could be used even at 250 mg/kg b.w. to complement currently available oral hypoglycemic drugs. The results suggest that microwave technology is a viable means for extracting valuable anti-diabetic components from medicinal plants. The reason for the observed differences in hypoglycemic activities by the various extract within the first 14 d and after the 21 d of experimental treatment could be due to the concentrations of the phyto-chemicals which vary in the extracts. It could also be possible that the different phytochemical exhibiting hypoglycemic effect have different rate of reaction in reducing the blood glucose level.

The main advantages of MAE over the conventional extraction techniques is that it reduces solvent consumption, it has a shorter operational time, modestly high recoveries, decent reproducibility and negligible



specimen control for extraction process<sup>[46]</sup>. Several biologically active compounds have been extracted by application of MAE, such as extraction of azadirachtin related limonoids from *Azadirachta indica* seed kernels<sup>[47]</sup>, extraction of artemisinin from *Artemisia annua*<sup>[48]</sup> and ginsenosides extraction from roots of *Panax ginseng*<sup>[44]</sup>, quercetin from herbal plant<sup>[22]</sup>. According to Pan *et al.*<sup>[49]</sup>, antioxidant activity of phenolic substances extricated from the peel of *Dimocarpus Longan* utilizing MAE was better than that of Soxhlet extraction. Besides, MAE of curcumin from *Curcuma longa* showed a better results and a higher extraction yield with noteworthy diminishing in the extraction time when compared to that of Soxhlet extraction, maceration and stirring extraction<sup>[50]</sup>. In the present study, there was higher yield in phenolics, saponins and tannins from both plants-VA and OG. This implies that MAE is a better technology for the extraction of these phytoconstituents. The soxhlet extraction technology showed a higher yield in alkaloid whereas maceration technology was best for the extraction of flavonoids.

We find the use of MAE leads to very fast extraction rate with high value of phytoconstituents compared to soxhlet and cold maceration technique. The findings obtained from the present research showed that the choice of extraction technology should be based primarily on the phytochemical entity of interest. For instance, with respect to the result from the present research, the use of soxhlet extraction technology would be recommended when alkaloid is the main phytoconstituent of interest, whereas, the cold maceration would be preferred for extraction of flavonoids. However, since there was no apparent destruction of any bioactive components by the extraction technologies studied, the MAE is recommended as the most suitable technology for routine extraction processes because it is faster, utilizes relatively less amount of solvent and saves more time. Nevertheless, since extraction efficiency differs from efficacy, no single method can be rated as best for extracting all forms of phyto-components. So, a further study on the isolation of the precise bioactive component(s) and its structural elucidation is recommended to ascertain the best technology for obtaining pure bioactive hypoglycemic compound(s) from medicinal plants.

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## COMMENTS

### Background

Although several methods are available for extraction of active phytoconstituents from medicinal plants. The technique that produces higher yield of specific

phytoconstituents has not been reported. The biological activities of these active phytoconstituents are known to be affected by the extraction protocol employed. To address these issues, this study examines three different procedures of extracting anti-diabetic substances from medicinal plants and evaluated their hypoglycemic activities.

### Research frontiers

There is active research in the field of investigation of a more effective extraction technique of active ingredient from medicinal plant. Hence, it is imperative to ascertain which extraction protocol produces a significant amount of specific phytoconstituents.

### Innovations and breakthroughs

The hypoglycemic activity of anti-diabetic plant is revealed in this study to be subject to the extraction method employed. For the first time, extraction method producing significantly high yield of specific phytoconstituents is presented.

### Applications

Since the biological efficacy of phytoconstituents is subject to the extraction protocol employed, the use of an appropriate extraction technique with respect to a specific active ingredient would enhance the process of drug development.

### Terminology

Phytoconstituents are chemical compounds that occur naturally in plants. Some of which are responsible for the medicinal effect of the plant.

### Peer-review

The authors provided the complete review of this issue. This manuscript provides the updated evidence to the readers.

## REFERENCES

- 1 Okoduwa SI, Umar IA, Ibrahim S, Bello F, Habila N. Age-dependent alteration of antioxidant defense system in hypertensive and type-2 diabetes patients. *J Diabetes Metab Disord* 2015; **14**: 32 [PMID: 25922827 DOI: 10.1186/s40200-015-0164-z]
- 2 Cheng Y, Malik U, Chang S. The risk factors of diabetic nephropathy in Taiwan, including old age, hypertension and aspirin therapy. *Int J Diabetes Dev C* 2013; **33** Suppl 2: 128 [DOI: 10.1007/s13410-013-0114-6]
- 3 Okoduwa SI, Umar IA, Ibrahim S, Bello F, Ndidi US. Socio-economic status of patients with type 2 diabetes and hypertension attending the Ahmadu Bello University Teaching Hospital, Zaria, North-West Nigeria. *Glob J Health Sci* 2015; **7**: 280-287 [PMID: 25560354 DOI: 10.5539/gjhs.v7n1p280]
- 4 American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2014; **37** Suppl 1: S81-S90 [PMID: 24357215 DOI: 10.2337/dc14-S081]
- 5 International Diabetes Federation. IDF Diabetes Atlas, 7th ed. Brussels, Belgium: International Diabetes Federation, 2015. Available from: URL: <http://www.diabetesatlas.org>
- 6 Fowler MJ. Diabetes Treatment, Part 2: Oral agents for glycemic management. *Clinical Diabetes* 2007; **25**: 131-134 [DOI: 10.2337/diaclin.25.4.131]
- 7 Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004; **27**: 1047-1053 [PMID: 15111519 DOI: 10.2337/diacare.27.5.1047]
- 8 Dey L, Attele AS, Yuan CS. Alternative therapies for type 2 diabetes. *Altern Med Rev* 2002; **7**: 45-58 [PMID: 11896745]
- 9 Fagbohun ED, Asare, RR and Egbebi, AO. Chemical composition and antimicrobial activities of *Urena lobata* L. (Malvaceae). *J Med Plants Res* 2012; **6** Suppl 12: 2256-2260 [DOI: 10.5897/JMPR09.233]
- 10 Farnsworth NR, Soejarto, DD. Global Importance of Medicinal Plants. In: Akerele O, Heywood V, Syngae H editors. The Conservation of Medicinal Plants Cambridge University Press,

- Cambridge, United Kingdom, 1991: 25-51 [DOI: 10.1017/CBO9780511753312.005]
- 11 **Handa SS.** An Overview of Extraction Techniques for Medicinal and Aromatic Plants. In: Handa et al., (eds): Extraction technologies for medicinal and aromatic plants, 1st ed, no. 66. Italy: United Nations Industrial Development Organization and the International Centre for Science and High Technology. Trieste, 2008: 21-25
  - 12 **Azwanida NN.** A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. *Med Aromat Plants* 2015; **4**: 196 [DOI: 10.4172/2167-0412.1000196]
  - 13 **Handa SS, Khanuja SPS, Longo G, Rakesh DD.** Extraction Technologies for Medicinal and Aromatic Plants, 1st ed, no. 66. Italy: United Nations Industrial Development Organization and the International Centre for Science and High Technology. Trieste, 2008
  - 14 **Prabhu KS, Lobo R, Shirwaikar AA, Shirwaikar A.** Ocimum gratissimum: A Review of its Chemical, Pharmacological and Ethnomedicinal Properties. *TOALTMED* 2009; **1**: 1-15 [DOI: 10.2174/1876391X00901010001]
  - 15 **Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H.** Phytochemical screening and extraction: a review. *Internationale Pharmaceutica Scientia* 2011; **1** Suppl 1: 98-106
  - 16 **Ncube NS, Afolayan AJ, Okoh AI.** Assessment techniques of antimicrobial property of natural compounds of plant origin. Current methods and future trend. *Afr J Biotechnol* 2008; **7** Suppl 12: 1797-1806 [DOI: 10.5897/AJB07.613]
  - 17 **Hijazi A, Bandar H, Rammal H, Hachem A, Saad Z, Badran B.** Techniques for the Extraction of Bioactive Compounds from Lebanese *Urtica dioica*. *AJPCT* 2013; **1**: Suppl 6: 507-513
  - 18 **Chemat F, Tomao V, Viot M.** In: Otles, S. (Ed.), Handbook of Food Analysis Instruments. Ultrasound-Assisted Extraction in Food Analysis. CRC Press, 2008: 85-94
  - 19 **Mandal V, Mohan Y, Hemalatha S.** Review Article Microwave Assisted Extraction an Innovative and Promising Extraction Tool for Medicinal Plant Research, Pharmacognosy Review. *PHCOG Rev* 2007; **1** Suppl 1: 7-18
  - 20 **Kenmogne SB, Ngassoum M, Tchatchueng JB, Vardamides JC, Dongmo.** Microwave Assisted Extraction of Analgesic Compounds of the Root of *Ximenia americana* (Olacaceae). *RJCS* 2014; **4** Suppl 7: 7-10
  - 21 **Savalia RV, Patel AP, Trivedi PT, Gohel HR, Khetani DB.** Rapid and Economic Synthesis of Schiff Base of Salicylaldehyde by Microwave Irradiation. *RJCS* 2013; **3** Suppl 10: 69-76
  - 22 **Chan CH, Yusoff R, Ngoh GC, Kung FW.** Extraction of anti-diabetic active ingredient, quercetin from herbal plant using microwave-assisted extraction (MAE) technique, International conference on Materials for Advanced Technologies. SUNTEC Singapore, 2011: KK-PO2-5 [DOI: 10.13140/2.1.3487.4885]
  - 23 **Tatke PA, Jirge, SS, Shukla TA.** An extraction procedure of scopoletin from *Convolvulus plaricuulis* (Shankhapushpi). *JMAPS* 2010; **31**: 126-126
  - 24 **Dhobi M, Mandal V, Hemalatha S.** Optimization of microwave assisted extraction of bioactive flavonolignan silybinin. *J Chem and Metrl* 2009; **3**: Suppl 1: 13-23
  - 25 **Mohammed YT, Okasha MA, Magaji RA, Yaro AH.** Effects of aqueous leaves extract of *Ocimum gratissimum* on blood glucose levels of streptozotocin-induced diabetic wistar rats. *Afr J Biotechnol* 2007; **6** Suppl 18: 2087-2090 [DOI: 10.5897/AJB2007.000-2323]
  - 26 **Abdulazeez MA, Ibrahim K, Bulus K, Babvoshia HB, Abdullahi Y.** Effect of combined use of *Ocimum gratissimum* and *Vernonia amygdalina* extract on the activity of angiotensin converting enzyme, hypolipidemic and antioxidant parameters in streptozotocin-induced diabetic rats. *AJBR* 2013; **7** Suppl 9: 165-173
  - 27 **Taoying Z, Denghong L, xingyuan L, Yunbo.** Hypoglycemic and hypolipidemic effects of flavonoids from lotus (*Nelumbo nucifera* Gaertn) leaf in diabetic mice. *J Med Plants Res* 2009; **3** Suppl 4: 290-293
  - 28 **Day C, Cartwright T, Provost J, Bailey CJ.** Hypoglycaemic effect of *Momordica charantia* extracts. *Planta Med* 1990; **56**: 426-429 [PMID: 2077547 DOI: 10.1055/s-2006-961003]
  - 29 **Francis G, Kerem Z, Makkar HP, Becker K.** The biological action of saponins in animal systems: a review. *Br J Nutr* 2002; **88**: 587-605 [PMID: 12493081 DOI: 10.1079/BJN2002725]
  - 30 **Okon UA, Owo DU, Udokang NE, Udobang JA, Ekpenyong CE.** Oral Administration of Aqueous Leaf Extract of *Ocimum Gratissimum* Ameliorates Polyphagia, Polydipsia and Weight Loss in Streptozotocin-Induced Diabetic Rats. *AJMSM* 2012; **2** Suppl 3: 45-49 [DOI: 10.5923/j.ajmms.20120203.04]
  - 31 **Modu S, Adeboye AE, Maisaratu A, Mubi BM.** Studies on the administration of *Vernonia amygdalina* Del. (Bitter leaf) and glucophage on blood glucose level of alloxan - Induced diabetic rats. *IJMPAM* 2013; **1** Suppl 1: 013-019
  - 32 **Proestos C, Komaitis M.** Application of microwave assisted extraction to the fast extraction of plant phenolic compounds. *LWT-Food Sci Technol* 2008; **41** Suppl 4: 652-659 [DOI: 10.1016/j.lwt.2007.04.013]
  - 33 **Gharekhani M, Rafiee Z, Ghorbani M, Jafari SM.** Open vessel microwave system for extraction of analytes from medicine plants. *Iran Patent* 2009: 59321
  - 34 **Asghari J, Ondruschka B, Mazaheritehrani M.** Extraction of bioactive chemical compounds from the medicinal Asian plants by microwave irradiation. *J Med Plants Res* 2011; **5** Suppl 4: 495-506
  - 35 **Evans WA.** Plants in African traditional medicines. An over view. In Trease and Evans Pharmacognosy (15th ed). India: Saunders in Print Elsevier, 2005: 448-491
  - 36 **Harbone JB.** Methods of extraction and isolation. In Phytochemical Methods. London: Chapman and Hall, 1998: 60-66
  - 37 **Markkar AOA, Goodchild AV.** Quantification of tannins. A laboratory manual. Aleppo Syria: International Centre for Agriculture Research in the dry areas. (ICARDA), Aleppo, Syria, 1996: 55
  - 38 **Bruneton J.** Pharmacognosy, phytochemistry, Medicinal plants. 2nd ed. Hamshire, UK: Intercept, 1999: 385-386
  - 39 **Harbone JB.** Phytochemistry Methods: a guide to modern techniques of plants analysis. London: Chapman and Hall, 1973: 267-270
  - 40 **Bohm BA, Koupai-Abyazani.** Flavonoids and condensed tannins from leaves of Hawaiian *Vacinium reticulatum* and *V. calycinum* (ericaceae). *Pacific Science* 1994; **48**: 458-463
  - 41 **Fermeglia M.** Role of Process Simulation in Extraction Technologies for Medicinal and Aromatic Plants. In: Extraction technologies for medicinal and aromatic plants, 2008
  - 42 **Vongsangnak W, Gua J, Chauvatcharin S, Zhong, JJ.** Toward Efficient Extraction of Notoginseng Saponins from Cultured Cells of Notoginseng. *Biochem Eng J* 2004; **18**: 115-120 [DOI: 10.1016/S1369-703X(03)00197-9]
  - 43 **Pan X, Niu G, Liu H.** Microwave-assisted extraction of tea polyphenols and tea caffeine from green tea leaves. *Chem Eng Process* 2003; **42** Suppl 2: 129-133 [DOI: 10.1016/S0255-2701(02)00037-5]
  - 44 **Shu YY, Ko MY and Chang YS.** Microwave assisted extraction of ginsenosides from ginseng root. *Microchem J* 2003; **74** Suppl 2: 131-139 [DOI: 10.1016/S0026-265X(02)00180-7]
  - 45 **Tatke P, Jaiswal Y.** An overview of Microwave Assisted Extraction and its Applications in Herbal Drugs Research. *Research Journal of Medicinal Plant* 2011; **5** Suppl 1: 21-31 [DOI: 10.3923/rjmp.2011.21.31]
  - 46 **Odabas HI, Koca I.** Application of response surface methodology for optimizing the recovery of phenolic compounds from hazelnut skin using different extraction methods. *Ind Crop Prod* 2016; **91**: 114-124 [DOI: 10.1016/j.indcrop.2016.05.033]
  - 47 **Dai J, Yaylayan, V, Raghavan G, Pare J.** Extraction and colorimetric determination of azadirachtin related limonoids in neem seed kernel. *J Agr Food Chem* 1999; **47**: 3738-3742 [DOI: 10.1021/jf990227h]
  - 48 **Hao JY, Han W, Huang SD, Xue BY, Deng X.** Microwave assisted extraction of artemisinin from *Artemisia annua* L. *Sep Purif Technol* 2002; **28** Suppl 3: 191-196 [DOI: 10.1016/S1383-5866(02)00043-6]
  - 49 **Pan Y, Wang K, Huang S, Wang H, Mu X, He C, Ji X, Zang J, Huang F.** Antioxidant activity of microwave assisted extract of

longan (*Dimorcarpus logan*) peel. *Food Chem* 2008; **106** Suppl 3: 1264-1270 [DOI: 10.1016/j.foodchem.2007.07.033]  
50 **Mandal V**, Mohan Y, Hemalatha S. Microwave assisted extraction

of curcumin by sample-solvent dual heating mechanism using Taguchi L9 orthogonal design. *J Pharmaceut Biomed* 2008; **46**: 322-327 [DOI: 10.1016/j.jpba.2007.10.020]

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Case Control Study

## Brain-derived neurotrophic factor plasma levels and premature cognitive impairment/dementia in type 2 diabetes

Blanca Murillo Ortiz, Joel Ramírez Emiliano, Edna Ramos-Rodríguez, Sandra Martínez-Garza, Hilda Macías-Cervantes, Sergio Solorio-Meza, Texar Alfonso Pereyra-Nobara

Blanca Murillo Ortiz, Edna Ramos-Rodríguez, Sandra Martínez-Garza, Hilda Macías-Cervantes, Sergio Solorio-Meza, Texar Alfonso Pereyra-Nobara, Unidad de Investigación en Epidemiología Clínica, Unidad Médica de Alta Especialidad No. 1 Bajío, Instituto Mexicano del Seguro Social, Guanajuato 37320, Mexico

Joel Ramírez Emiliano, Departamento de Ciencias Médicas, División Ciencias de la Salud, Universidad de Guanajuato, Guanajuato 37320, Mexico

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**Correspondence to:** Blanca Murillo Ortiz, PhD, Unidad de Investigación en Epidemiología Clínica, Unidad Médica de Alta Especialidad No. 1 Bajío, Instituto Mexicano del Seguro Social, López Mateos Esquina Insurgentes s/n Colonia Los Paraísos,

León, Guanajuato 37320, Mexico. [bomo907@hotmail.com](mailto:bomo907@hotmail.com)  
Telephone: +52-477-7183039  
Fax: +52-477-7183039

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### Abstract

#### AIM

To assess the relationship of brain-derived neurotrophic factor (BDNF) with cognitive impairment in patients with type 2 diabetes.

#### METHODS

The study included 40 patients with diabetes mellitus type 2 (DM2), 37 patients with chronic kidney disease in hemodialysis hemodialysis therapy (HD) and 40 healthy subjects. BDNF in serum was quantified by ELISA. The Folstein Mini-Mental State Examination was used to evaluate cognitive impairment.

#### RESULTS

The patients with DM2 and the patients in HD were categorized into two groups, with cognitive impairment and without cognitive impairment. The levels of BDNF showed significant differences between patients with DM2 ( $43.78 \pm 9.05$  vs  $31.55 \pm 10.24$ ,  $P = 0.005$ ). There were no differences between patients in HD ( $11.39 \pm 8.87$  vs  $11.11 \pm 10.64$ ,  $P = 0.77$ ); interestingly, ferritin levels were higher in patients with cognitive impairment ( $1564 \pm 1335$  vs  $664 \pm 484$ ,  $P = 0.001$ ). The comparison



of BDNF values, using a Kruskal Wallis test, between patients with DM2, in HD and healthy controls showed statistical differences ( $P < 0.001$ ).

## CONCLUSION

Low levels of BDNF are associated with cognitive impairment in patients with DM2. The decrease of BDNF occurs early and progressively in patients in HD.

**Key words:** Diabetes mellitus type 2; Hemodialysis; Brain-derived neurotrophic factor; Folstein mini-mental; Premature cognitive impairment

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**Core tip:** The objective was to compare serum levels of brain-derived neurotrophic factor (BDNF) between patients with and without cognitive impairment, patients with diabetes mellitus type 2 (DM2) and chronic kidney disease patients on hemodialysis, in order to increase our knowledge on the possible role of BDNF in early cognitive impairment in DM2. We found differences in serum BDNF levels; they were lowest in patients with DM2 with cognitive impairment. In patients on hemodialysis, serum BDNF levels were lower than in patients with DM2 and healthy controls and ferritin levels were higher in patients with cognitive impairment.

Murillo Ortiz B, Ramírez Emiliano J, Ramos-Rodríguez E, Martínez-Garza S, Macías-Cervantes H, Solorio-Meza S, Pereyra-Nobara TA. Brain-derived neurotrophic factor plasma levels and premature cognitive impairment/dementia in type 2 diabetes. *World J Diabetes* 2016; 7(20): 615-620 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v7/i20/615.htm> DOI: <http://dx.doi.org/10.4239/wjd.v7.i20.615>

## INTRODUCTION

Brain-derived neurotrophic factor (BDNF) is a growth factor that belongs to the neurotrophin family; its mature isoform binds specifically to the tropomyosin receptor kinase B, a tyrosine kinase receptor, whereas the precursor pro-BDNF binds to the pan-neurotrophin receptor p75NTR; each mediate different neurotrophic signals<sup>[1,2]</sup>. BDNF is also important for learning and memory processes, as it induces long-term potentiation in hippocampus and structural changes in synapses.

A positive correlation between brain BDNF concentration and cognitive performance has been described, while decreased BDNF production has been proposed as one possible pathogenetic factor for Alzheimer's disease and major depression<sup>[3,4]</sup>. Interestingly, plasma BDNF levels are decreased in patients with diabetes mellitus type 2 (DM2) and have been inversely correlated with plasma glucose and insulin resistance as assessed by homeostatic model assessment. Moreover, the output of plasma BDNF from the human brain is abrogated by hyperglycemia, but

it is not regulated by hyperinsulinemia<sup>[5]</sup>.

Zhen *et al*<sup>[6]</sup> found both lower serum BDNF levels and impaired cognitive functions in diabetic patients compared to controls; furthermore, a positive relationship between serum BDNF and delayed memory was observed in diabetic patients, suggesting a role for BDNF in cognitive deficit associated with DM2.

A longer duration of DM2 has been associated with a major risk of chronic kidney disease (CKD), and has been considered a possible new determinant of cognitive decline and dementia<sup>[7]</sup>. Most recent prospective studies have found an association between CKD and cognitive decline<sup>[8-11]</sup>. The Health, Aging, and Body Composition Study demonstrated that more advanced stages of CKD are associated with an increased risk for cognitive impairment<sup>[12]</sup>. BDNF plays a critical role in the functioning of the brain<sup>[13-20]</sup>. It has been observed that the concentration of serum BDNF reflects the changes in brain BDNF levels<sup>[21-23]</sup>; therefore, measuring the concentration of serum BDNF can be used to monitor its changes in the brain<sup>[24]</sup>. It was recently demonstrated that BDNF stimulates the production of prostacyclin in cerebral arteries<sup>[25]</sup>; it plays an important role in endothelium-dependent relaxation and has also antiplatelet, vasculoprotective, cardioprotective and anti-atherogenic properties<sup>[26-28]</sup>. Zoladz *et al*<sup>[29]</sup> demonstrated that the decrease in serum BDNF levels after hemodialysis is accompanied by elevated levels of F-isoprostanes and decreased plasma total antioxidant capacity, which might be caused by the increase in oxidative stress induced by hemodialysis.

The aim of the present study was to compare serum levels of BDNF and the results of the mini mental state examination between patients with DM2 and patients with CKD on hemodialysis, in order to obtain more information on the possible role of BDNF in premature cognitive impairment/dementia in type 2 diabetes. We also investigated whether BDNF predicted premature cognitive impairment, and if it was associated with any clinical parameters in a group of patients with chronic kidney disease.

## MATERIALS AND METHODS

A cross-sectional study was carried out in three groups of patients from the Unidad Médica de Alta Especialidad (UMAE) No. 1 Bajío, Instituto Mexicano del Seguro Social (IMSS), León, Guanajuato, México; the patients were matched by age.

### Patients with DM2

We selected 37 diabetic male patients, aged 39-59 year (mean age  $50.57 \pm 5.9$  year) with a history of DM2 with a duration of  $14.3 \pm 6.22$  year.

### Patients with chronic kidney disease on hemodialysis

We investigated 40 men, aged 18-67 year (mean  $\pm$  SD, mean age  $42.30 \pm 12.8$  years), with chronic kidney disease, who had started hemodialysis therapy (HD).

**Table 1** Clinical characteristics of hemodialysis patients

Baseline characteristics	HD patients with cognitive impairment ( <i>n</i> = 17)	HD patients without cognitive impairment ( <i>n</i> = 23)	<i>P</i>
Age (yr)	51.88 ± 12.81	42.30 ± 12.87	0.02
Duration of hemodialysis (mon)	41.29 ± 42.01	32.08 ± 36.76	0.13
BDNF (ng/mL)	11.39 ± 8.87	11.11 ± 10.64	0.77
Creatinine (mg/dL)	8.90 ± 1.90	9.21 ± 2.61	0.68
Urea (mg/dL)	128.68 ± 54.23	127.74 ± 51.54	0.77
Hemoglobin (g/dL)	12.13 ± 1.38	11.53 ± 1.92	0.51
Ferritin (ng/mL)	1564 ± 1335.05	664.22 ± 484.99	0.001
Mini-mental state examination	19.58 ± 3.24	26.08 ± 1.50	0.0001

BDNF: Brain-derived neurotrophic factor; HD: Hemodialysis therapy.

We excluded patients older than 69 years of age and those with acute infectious diseases, psychiatric diseases or severe liver dysfunction. Baseline demographic and clinical data such as age, primary cause of renal disease and current medications were collected from the patients' records.

### Healthy control subjects

The control group was formed by forty healthy male volunteers from the same demographic group as the patients; they were aged 39-60 years (mean age 42 ± 2.2 year) and received annual health examinations.

Fasting blood samples were collected from patients and healthy controls at 8 am. Serum BDNF concentrations were determined by ELISA using the Human BDNF Quantikine Kit. The concentrations of ferritin in serum and other biochemical parameters were measured at the Central Clinical Laboratory. A neurological assessment was performed before each hemodialysis session (Mini Mental).

The study was performed in accordance with the Declaration of Helsinki of the World Medical Association and was approved by the local Bioethics Committee of the UMAE No. 1 Bajío, IMSS, León, Guanajuato, México. All patients signed an informed consent form for this investigation.

### Statistical analysis

The statistical analysis was performed using Microsoft Excel and Statistica software. The statistical significance of the differences observed between patients and controls was assessed using two-tailed *t*-test,  $\chi^2$  and Kruskal-Wallis (*P* < 0.05).

## RESULTS

The patients with DM2 and on hemodialysis were categorized according to the score obtained in the Folstein Mini-Mental State Examination into a group with cognitive impairment and a group without cognitive impairment. The group of patients with type 2 diabetes and cognitive impairment had 19 patients and the group without cognitive impairment had 18 patients. The average age was 50.57 ± 5.9 years with a history of type 2 diabetes mellitus with a duration of 14.3 ± 6.22 years. We were able to analyze the differences between patients with

and without cognitive impairment (Table 1). We observed significant differences in the levels of glycated Hb, which were higher in patients with cognitive impairment (8.36 ± 1.52 vs 7.33 ± 1.42 *P* = 0.02). There were also differences in the duration of diabetes; patients with cognitive impairment had more years of DM2 (14.31 ± 6.22 vs 9.05 ± 4.64, *P* = 0.007). The values of serum BDNF also showed significant differences between patients with and without cognitive impairment (31.55 ± 10.24 vs 43.78 ± 9.05, *P* = 0.005).

The group of patients on HD with cognitive impairment had 17 patients; the group without cognitive impairment had 23 patients. The average age of the patients was 42.30 ± 12.8 years. The most common cause of renal failure was diabetes mellitus (45%), followed by glomerulonephritis (20%), renal hypoplasia (15%), hypertension (10%) and other causes (10%). Sixty-five point seven percent of the patients had been subjected to a vascular access procedure using a catheter, while only 31.2% had an arteriovenous fistula. There were also significant differences in patients with chronic renal disease on replacement therapy with hemodialysis between those with and without cognitive impairment (Table 2). Ferritin levels were higher in patients with cognitive impairment (1564 ± 1335 vs 664 ± 484, *P* = 0.001), in contrast to serum levels of BDNF (11.39 ± 8.87 vs 11.11 ± 10.64, *P* = 0.77); however, both groups of patients on hemodialysis had lower levels than healthy controls.

The serum BDNF levels of healthy control subjects were 39.36 ± 8.9 ng/mL. The comparison of BDNF levels, using a Kruskal Wallis test, between patients with DM2, HD and healthy controls showed statistically significant differences (*P* < 0.001) (Figure 1).

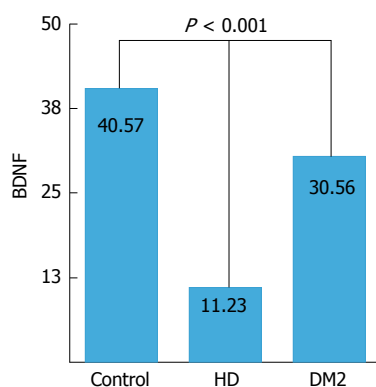
## DISCUSSION

Most recent prospective studies associate chronic kidney disease with cognitive impairment. There has been a significant increase in the prevalence of chronic degenerative diseases worldwide; thus, there is a particular interest in learning how to modify the conditions that cause cognitive decline and dementia. DM2 has been strongly associated with an increased loss of cognitive functions. A recent cohort study showed that high glucose levels may be a risk factor for dementia and

**Table 2** Clinical characteristics of diabetes mellitus type 2 patients

Baseline characteristics	DM2 patients with cognitive impairment ( <i>n</i> = 19)	DM2 patients without cognitive impairment ( <i>n</i> = 18)	<i>P</i>
Age (yr)	50.57 ± 5.90	54.05 ± 3.63	0.06
Duration of DM2 (yr)	14.31 ± 6.22	9.05 ± 4.64	0.007
BDNF (ng/mL)	31.55 ± 10.24	43.78 ± 9.05	0.005
Glucose (mg/dL)	177 ± 64.91	138 ± 43.90	0.07
Glycated hemoglobin (HBA1c) (%)	8.36 ± 1.52	7.33 ± 1.42	0.02
Minimetal state examination	20.26 ± 2.15	25.44 ± 1.50	0.0001

BDNF: Brain-derived neurotrophic factor; DM2: Diabetes mellitus type 2.



**Figure 1** Difference between serum brain-derived neurotrophic factor levels between control subjects and patients in hemodialysis therapy patients with diabetes mellitus type 2. BDNF: Brain-derived neurotrophic factor; HD: Hemodialysis therapy; DM2: Diabetes mellitus type 2.

that the combination of DM2 and hypertension greatly increase the risk of cognitive impairment. Beside vascular factors, other risk factors include the formation of advanced glycosylation products, oxidative inflammation and stress, alterations in the hypothalamic-pituitary-adrenal axis and cortisol levels<sup>[30]</sup>, and abnormalities in insulin secretion and signaling that promote cerebral amyloidosis.

The analysis of the relationship between serum ferritin and Mini-Mental scores in HD patients showed a significant difference between serum ferritin levels and the presence of cognitive impairment according to the Folstein test. Therefore, we can say that a higher iron overload corresponds to greater cognitive impairment.

Becerril-Ortega *et al.*<sup>[31]</sup> analyzed the relationship between iron and neurodegenerative diseases (especially Alzheimer's disease) that affect cognitive impairment in a transgenic mouse model; they observed that iron interferes with the processing of the amyloid precursor protein (APP), neuronal signaling and cognitive behavior. The proposed mechanism is that iron overload increases the production of amyloidogenic KPI-APP and amyloid beta; this is mediated by N-methyl-D-aspartate receptors (NMDAR), mainly GluN2B, which is overexpressed. These data suggest that the damage induced by iron overload through APP accelerates cognitive impairment due to excessive extrasynaptic NMDAR activity 30, causing a significant memory and learning deficit, and inhibiting synaptic plasticity, mitochondrial dysfunction and neuronal apoptosis, which can lead to neurodegeneration.

This is also supported by a study that showed evidence of iron overload in brain structures such as the putamen, dentate nucleus, substantia nigra and red nucleus of patients with beta-thalassemia<sup>[32]</sup>. Another group of patients with thalassemia also showed iron overload and increased oxidative damage<sup>[33]</sup>. Blasco *et al.*<sup>[34]</sup> found a significant positive association between obesity, insulin resistance and iron overload in the caudate nucleus, hypothalamus and hippocampus, and poor cognitive performance. Furthermore, it has been shown that iron overload causes oxidative stress *in vitro*<sup>[35]</sup> and can affect the hematopoiesis of bone marrow in mice by increasing oxidative stress<sup>[36]</sup>.

Although there are multiple factors that influence cognitive impairment, several studies have shown an association with circulating levels of BDNF<sup>[37,38]</sup>, and have suggested a synergistic effect between the presence of dementia and BDNF levels in DM2<sup>[39]</sup>. Our study found this association and also that patients on HD had increased oxidative stress, probably induced by iron overload, which was evidenced by elevated levels of ferritin. This was significantly associated with greater cognitive impairment and with serum BDNF levels well below the levels found in patients with type 2 diabetes mellitus and healthy controls.

One of the most common advanced complications of DM2 is CKD. The progressive loss of renal function could make it necessary for the patient to receive renal replacement therapy such as hemodialysis, and the progressive loss of circulating levels of BDNF should be prevented in patients with DM2 to avoid premature cognitive decline. There have been several experimental studies with curcumin<sup>[40]</sup> and resveratrol, both of which increase serum BDNF levels. Curcumin has an antidepressant effect, mediated by its antioxidant activity and up-regulation of phosphor Akt and mTOR levels in the hippocampus and prefrontal cortex<sup>[41]</sup>. Resveratrol has antidepressant-like effects, mediated in part by the normalization of serum corticosterone levels and the up-regulation of Perk, pCREB and BDNF levels in the hippocampus and amygdala<sup>[42]</sup>. This is an alternative that should be investigated further in future randomized clinical trials.

## COMMENTS

### Background

Diabetes mellitus type 2 (DM2) has been strongly associated with an increased

loss of cognitive functions, while decreased brain-derived neurotrophic factor (BDNF) production has been proposed as one possible pathogenetic factor for premature cognitive impairment/dementia. A longer duration of DM2 has been associated with a major risk of chronic kidney disease, and has been considered a possible new determinant of cognitive decline and dementia.

### Research frontiers

The analysis of the relationship between serum ferritin and Mini-Mental scores in hemodialysis therapy (HD) patients showed a significant difference between serum ferritin levels and the presence of cognitive impairment according to the Folstein test. Therefore, the authors can say that a higher iron overload corresponds to greater cognitive impairment, and it is of interest which factors modify the decrement of BDNF production. Measuring the concentration of serum BDNF can be used to monitor its changes in the brain, in order to influence the course of the disease.

### Innovations and breakthroughs

The authors confirm the serum BDNF levels between patients with DM2, HD and healthy controls showed statistically significant differences. Ferritin levels were higher in patients in HD with cognitive impairment, is a breakthrough in the understanding of the factors contributing to the loss of BDNF and cognitive impairment.

### Applications

Monitoring levels of BDNF can prevent cognitive decline implementing new measures such as the use of antioxidants proposed recently and currently under research.

### Peer-review

The study is original and evaluates the cognitive impairment in diabetes mellitus in relationship with the brain-derived neurotrophic factor plasma levels and ferritin. The article has interest and likes suitable for the publication in the Journal.

## REFERENCES

- Cohen-Cory S, Kidane AH, Shirkey NJ, Marshak S. Brain-derived neurotrophic factor and the development of structural neuronal connectivity. *Dev Neurobiol* 2010; **70**: 271-288 [PMID: 20186709 DOI: 10.1002/dneu.20774]
- Zoladz JA, Pilc A. The effect of physical activity on the brain derived neurotrophic factor: from animal to human studies. *J Physiol Pharmacol* 2010; **61**: 533-541 [PMID: 21081796]
- Peng S, Wu J, Mufson EJ, Fahnstock M. Precursor form of brain-derived neurotrophic factor and mature brain-derived neurotrophic factor are decreased in the pre-clinical stages of Alzheimer's disease. *J Neurochem* 2005; **93**: 1412-1421 [PMID: 15935057 DOI: 10.1111/j.1471-4159.2005]
- Connor B, Young D, Yan Q, Faull RL, Synek B, Dragunow M. Brain-derived neurotrophic factor is reduced in Alzheimer's disease. *Brain Res Mol Brain Res* 1997; **49**: 71-81 [PMID: 9387865]
- Chaldakov GN, Tonchev AB, Manni L, Hristova MG, Nikolova V, Fiore M, Vyagova D, Peneva VN, Aloe L. Comment on: Krabbe KS, Nielsen AR, Krogh-Madsen R et al (2007) Brain-derived neurotrophic factor (BDNF) and type 2 diabetes. *Diabetologia* 50: 431-438. *Diabetologia* 2007; **50**: 1781-1782 [PMID: 17546439 DOI: 10.1007/s00125-007-0706-0]
- Zhen YF, Zhang J, Liu XY, Fang H, Tian LB, Zhou DH, Kosten TR, Zhang XY. Low BDNF is associated with cognitive deficits in patients with type 2 diabetes. *Psychopharmacology (Berl)* 2013; **227**: 93-100 [PMID: 23263460 DOI: 10.1007/s00213-012-2942-3]
- Khatri M, Nickolas T, Moon YP, Paik MC, Rundek T, Elkind MS, Sacco RL, Wright CB. CKD associates with cognitive decline. *J Am Soc Nephrol* 2009; **20**: 2427-2432 [PMID: 19729443 DOI: 10.1681/ASN.2008101090]
- Buchman AS, Tanne D, Boyle PA, Shah RC, Leurgans SE, Bennett DA. Kidney function is associated with the rate of cognitive decline in the elderly. *Neurology* 2009; **73**: 920-927 [PMID: 19657107 DOI: 10.1212/WNL.0b013e3181b72629]
- Sasaki Y, Marioni R, Kasai M, Ishii H, Yamaguchi S, Meguro K. Chronic kidney disease: a risk factor for dementia onset: a population-based study. The Osaka-Tajiri Project. *J Am Geriatr Soc* 2011; **59**: 1175-1181 [PMID: 21668914 DOI: 10.1111/j.1532-5415.2011.03477.x]
- Davey A, Elias MF, Robbins MA, Seliger SL, Dore GA. Decline in renal functioning is associated with longitudinal decline in global cognitive functioning, abstract reasoning and verbal memory. *Nephrol Dial Transplant* 2013; **28**: 1810-1819 [PMID: 23166308 DOI: 10.1093/ndt/gfs470]
- Darsie B, Shlipak MG, Sarnak MJ, Katz R, Fitzpatrick AL, Odden MC. Kidney function and cognitive health in older adults: the Cardiovascular Health Study. *Am J Epidemiol* 2014; **180**: 68-75 [PMID: 24844846 DOI: 10.1093/aje/kwu102]
- Kurella M, Chertow GM, Fried LF, Cummings SR, Harris T, Simonsick E, Satterfield S, Ayonayon H, Yaffe K. Chronic kidney disease and cognitive impairment in the elderly: the health, aging, and body composition study. *J Am Soc Nephrol* 2005; **16**: 2127-2133 [PMID: 15888561 DOI: 10.1681/ASN.2005010005]
- Binder DK, Scharfman HE. Brain-derived neurotrophic factor. *Growth Factors* 2004; **22**: 123-131 [PMID: 15518235]
- Monteggia LM, Barrot M, Powell CM, Berton O, Galanis V, Gemelli T, Meuth S, Nagy A, Greene RW, Nestler EJ. Essential role of brain-derived neurotrophic factor in adult hippocampal function. *Proc Natl Acad Sci USA* 2004; **101**: 10827-10832 [PMID: 15249684 DOI: 10.1073/pnas.0402141101]
- McAllister AK, Katz LC, Lo DC. Neurotrophins and synaptic plasticity. *Annu Rev Neurosci* 1999; **22**: 295-318 [PMID: 10202541 DOI: 10.1146/annurev.neuro.22.1.295]
- Figurov A, Pozzo-Miller LD, Olafsson P, Wang T, Lu B. Regulation of synaptic responses to high-frequency stimulation and LTP by neurotrophins in the hippocampus. *Nature* 1996; **381**: 706-709 [PMID: 8649517 DOI: 10.1038/381706a0]
- Kafitz KW, Rose CR, Thoenen H, Konnerth A. Neurotrophin-evoked rapid excitation through TrkB receptors. *Nature* 1999; **401**: 918-921 [PMID: 10553907 DOI: 10.1038/44847]
- Alonso M, Vianna MR, Izquierdo I, Medina JH. Signaling mechanisms mediating BDNF modulation of memory formation in vivo in the hippocampus. *Cell Mol Neurobiol* 2002; **22**: 663-674 [PMID: 12585686]
- Kossel AH, Cambridge SB, Wagner U, Bonhoeffer T. A caged Ab reveals an immediate/instructive effect of BDNF during hippocampal synaptic potentiation. *Proc Natl Acad Sci USA* 2001; **98**: 14702-14707 [PMID: 11724927 DOI: 10.1073/pnas.251326998]
- Lee JL, Everitt BJ, Thomas KL. Independent cellular processes for hippocampal memory consolidation and reconsolidation. *Science* 2004; **304**: 839-843 [PMID: 15073322 DOI: 10.1126/science.1095760]
- Karege F, Schwald M, Cisse M. Postnatal developmental profile of brain-derived neurotrophic factor in rat brain and platelets. *Neurosci Lett* 2002; **328**: 261-264 [PMID: 12147321]
- Sartorius A, Hellweg R, Litzke J, Vogt M, Dormann C, Vollmayr B, Danker-Hopfe H, Gass P. Correlations and discrepancies between serum and brain tissue levels of neurotrophins after electroconvulsive treatment in rats. *Pharmacopsychiatry* 2009; **42**: 270-276 [PMID: 19924587 DOI: 10.1055/s-0029-1224162]
- Blugeot A, Rivat C, Bouvier E, Molet J, Mouchard A, Zeau B, Bernard C, Benoliel JJ, Becker C. Vulnerability to depression: from brain neuroplasticity to identification of biomarkers. *J Neurosci* 2011; **31**: 12889-12899 [PMID: 21900567 DOI: 10.1523/JNEUROSCI.1309-11.2011]
- Nakahashi T, Fujimura H, Altar CA, Li J, Kambayashi J, Tandon NN, Sun B. Vascular endothelial cells synthesize and secrete brain-derived neurotrophic factor. *FEBS Lett* 2000; **470**: 113-117 [PMID: 10734218]
- Santhanam AV, Smith LA, Katusic ZS. Brain-derived neurotrophic factor stimulates production of prostacyclin in cerebral arteries. *Stroke* 2010; **41**: 350-356 [PMID: 20019327 DOI: 10.1161/STROKEAHA.109.564492]
- Gryglewski RJ. Prostaglandins, platelets, and atherosclerosis. *CRC Crit Rev Biochem* 1980; **7**: 291-338 [PMID: 6771102]
- Grosser T, Fries S, FitzGerald GA. Biological basis for the cardio-



- vascular consequences of COX-2 inhibition: therapeutic challenges and opportunities. *J Clin Invest* 2006; **116**: 4-15 [PMID: 16395396 DOI: 10.1172/JCI27291]
- 28 **Chlopicki S**, Swies J, Mogielnicki A, Buczek W, Bartus M, Lomnicka M, Adamus J, Gebicki J. 1-Methylnicotinamide (MNA), a primary metabolite of nicotinamide, exerts anti-thrombotic activity mediated by a cyclooxygenase-2/prostacyclin pathway. *Br J Pharmacol* 2007; **152**: 230-239 [PMID: 17641676 DOI: 10.1038/sj.bjp.0707383]
- 29 **Zoladz JA**, Śmigielski M, Majerczak J, Nowak ŁR, Zapart-Bukowska J, Smoleński O, Kulpa J, Duda K, Drzewińska J, Bartosz G. Hemodialysis decreases serum brain-derived neurotrophic factor concentration in humans. *Neurochem Res* 2012; **37**: 2715-2724 [PMID: 22903469 DOI: 10.1007/s11064-012-0862-6]
- 30 **Passaro A**, Dalla Nora E, Morieri ML, Soavi C, Sanz JM, Zurlo A, Fellin R, Zuliani G. Brain-derived neurotrophic factor plasma levels: relationship with dementia and diabetes in the elderly population. *J Gerontol A Biol Sci Med Sci* 2015; **70**: 294-302 [PMID: 24621946 DOI: 10.1093/gerona/glu028]
- 31 **Becerril-Ortega J**, Bordji K, Fréret T, Rush T, Buisson A. Iron overload accelerates neuronal amyloid- $\beta$  production and cognitive impairment in transgenic mice model of Alzheimer's disease. *Neurobiol Aging* 2014; **35**: 2288-2301 [PMID: 24863668 DOI: 10.1016/j.neurobiolaging.2014.04.019]
- 32 **Qiu D**, Chan GC, Chu J, Chan Q, Ha SY, Moseley ME, Khong PL. MR quantitative susceptibility imaging for the evaluation of iron loading in the brains of patients with  $\beta$ -thalassemia major. *AJNR Am J Neuroradiol* 2014; **35**: 1085-1090 [PMID: 24578278 DOI: 10.3174/ajnr.A3849]
- 33 **Karakas Z**, Yilmaz Y, Celik DD, Annayev A, Demirel S, Kuruca SE. Total oxidant and antioxidant capacity in patients with transfusion dependent and nondependent beta thalassemia. Proceedings of the 57<sup>th</sup> ASH Annual Meeting Abstracts, 2015 Dec Vol 126, Issue 23: 4573
- 34 **Blasco G**, Puig J, Daunis-I-Estadella J, Molina X, Xifra G, Fernández-Aranda F, Pedraza S, Ricart W, Portero-Otín M, Fernández-Real JM. Brain iron overload, insulin resistance, and cognitive performance in obese subjects: a preliminary MRI case-control study. *Diabetes Care* 2014; **37**: 3076-3083 [PMID: 25125507 DOI: 10.2337/dc14-0664]
- 35 **Lu WY**, Zhao MF, Chai X, Meng JX, Xie F, Mu J, Zhu HB, Xu XN, Xiao X, Deng Q, Ma L. Iron Overload Impairs Hematopoiesis by Damaging MSCs Through ROS Signaling Pathway. Proceedings of the 54<sup>th</sup> ASH Annual Meeting Abstracts, 2012 Nov 16, Vol 120, Issue 21: 92-5172
- 36 **Chai X**, Li D, Cao X, Zhang Y, Mu J, Lu W, Xiao X, Li C, Meng J, Chen J, Li Q, Wang J, Meng A, Zhao M. ROS-mediated iron overload injures the hematopoiesis of bone marrow by damaging hematopoietic stem/progenitor cells in mice. *Sci Rep* 2015; **5**: 10181 [PMID: 25970748 DOI: 10.1038/srep10181]
- 37 **Ono M**, Ichihara J, Nonomura T, Itakura Y, Taiji M, Nakayama C, Noguchi H. Brain-derived neurotrophic factor reduces blood glucose level in obese diabetic mice but not in normal mice. *Biochem Biophys Res Commun* 1997; **238**: 633-637 [PMID: 9299565 DOI: 10.1006/bbrc.1997.7220]
- 38 **Ott A**, Stolk RP, van Harskamp F, Pols HA, Hofman A, Breteler MM. Diabetes mellitus and the risk of dementia: The Rotterdam Study. *Neurology* 1999; **53**: 1937-1942 [PMID: 10599761]
- 39 **Gregg EW**, Yaffe K, Cauley JA, Rolka DB, Blackwell TL, Narayan KM, Cummings SR. Is diabetes associated with cognitive impairment and cognitive decline among older women? Study of Osteoporotic Fractures Research Group. *Arch Intern Med* 2000; **160**: 174-180 [PMID: 10647755]
- 40 **Franco-Robles E**, Campos-Cervantes A, Murillo-Ortiz BO, Segovia J, López-Briones S, Vergara P, Pérez-Vázquez V, Solís-Ortiz MS, Ramírez-Emiliano J. Effects of curcumin on brain-derived neurotrophic factor levels and oxidative damage in obesity and diabetes. *Appl Physiol Nutr Metab* 2014; **39**: 211-218 [PMID: 24476477 DOI: 10.1139/apnm-2013-0133]
- 41 **Liu D**, Wang Z, Gao Z, Xie K, Zhang Q, Jiang H, Pang Q. Effects of curcumin on learning and memory deficits, BDNF, and ERK protein expression in rats exposed to chronic unpredictable stress. *Behav Brain Res* 2014; **271**: 116-121 [PMID: 24914461 DOI: 10.1016/j.bbr.2014.05.068]
- 42 **Liu D**, Xie K, Yang X, Gu J, Ge L, Wang X, Wang Z. Resveratrol reverses the effects of chronic unpredictable mild stress on behavior, serum corticosterone levels and BDNF expression in rats. *Behav Brain Res* 2014; **264**: 9-16 [PMID: 24503118 DOI: 10.1016/j.bbr.2014.01.039]

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## Retrospective Study

# Double diabetes in Saudi Arabia: A new entity or an underestimated condition

Rim Braham, Aus Alzaid, Asirvatham Alwin Robert, Muhammad Mujammami, Rania Ahmad Ahmad, Monther Zitouni, Samia Hasan Sobki, Mohamed Abdulaziz Al Dawish

Rim Braham, Aus Alzaid, Asirvatham Alwin Robert, Rania Ahmad Ahmad, Mohamed Abdulaziz Al Dawish, Department of Endocrinology and Diabetes, Prince Sultan Military Medical City, Riyadh 11159, Saudi Arabia

Muhammad Mujammami, Endocrinology and Diabetes Unit, Department of Medicine, King Khalid University Hospital, King Saud University, Riyadh 11461, Saudi Arabia

Monther Zitouni, Samia Hasan Sobki, Department of Pathology, Prince Sultan Military Medical City, Riyadh 11159, Saudi Arabia

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**Correspondence to:** Dr. Rim Braham, Department of Endo-

crinology and Diabetes, Prince Sultan Military Medical City, P.O. Box 7897, Riyadh 11159, Saudi Arabia. [braham\\_rim@yahoo.fr](mailto:braham_rim@yahoo.fr)  
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## Abstract

### AIM

To determine the clinical and biological characteristics of double diabetes (DD) among young people in Saudi Arabia.

### METHODS

This was a retrospective descriptive chart review study including 312 young newly diagnosed diabetic patients (aged 12-20 years), whom were admitted over a five year period (January 2009 to December 2013). Family history of diabetes mellitus (DM) (first degree), physical body mass index (BMI), acanthosis nigricans, history of auto-immune disease and laboratory information for glycosylated hemoglobin, basal C peptide level and diabetes autoantibody response (anti-GAD, anti-IA2 and anti-ICA) were collected from medical report. A mean follow-up of 3 years for these patients was performed.

### RESULTS

Patients were categorized into 4 groups, based on the autoantibody response (Ab+ or Ab-) and C-peptide secretion ( $\beta$ + for fasting level 0.4-2.1 ng/mL and  $\beta$ - if < 0.4 ng/mL). Group1 (type 1a): Ab+  $\beta$ - (21%), group 2 (type 1b): Ab-  $\beta$ - (9%), group 3 (DD): Ab+  $\beta$ + (31%)

and group 4 (classic type 2 DM): Ab-  $\beta$ + (39%). The mean age of the DD patients in our study was  $15.1 \pm 6.4$  years. A total of 41% of the study population presented with diabetic ketoacidosis and 61% of the study population presented with positive family history of DM. The mean BMI was  $26.8 \text{ kg/m}^2$  with 64% of overweight or obese patients. Ninety two percent of the patients were started on insulin at the time of diagnosis. During a mean follow-up of 3 years, only 32% of the patients with DD required insulin and 78% were on metformin alone or with insulin.

### CONCLUSION

Our findings enable us to arrive at the conclusion that almost one-third of the young Saudi diabetic patients reveal atypical forms of DM (double diabetes) expressing features resulting from both T1D and T2D.

**Key words:** Double diabetes; Therapeutic approaches; Hybrid diabetes; Autoantibody response; Saudi Arabia

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**Core tip:** Almost one-third of the young Saudi diabetic patients reveal atypical forms of double diabetes (DD) expressing features resulting from both type 1 diabetes and type 2 diabetes. Therefore, identification of DD patients becomes important as this will give direction for the selection of the most apt diagnostic and therapeutic lines of treatment.

Braham R, Alzaid A, Robert AA, Mujammami M, Ahmad RA, Zitouni M, Sobki SH, Al Dawish MA. Double diabetes in Saudi Arabia: A new entity or an underestimated condition. *World J Diabetes* 2016; 7(20): 621-626 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v7/i20/621.htm> DOI: <http://dx.doi.org/10.4239/wjd.v7.i20.621>

### INTRODUCTION

Almost one-third of the young Saudi diabetic patients reveal atypical forms of double diabetes (DD) expressing features resulting from both type 1 diabetes and type 2 diabetes. Therefore, identification of DD patients becomes important as this will give direction for the selection of the most apt diagnostic and therapeutic lines of treatment.

Diabetes mellitus (DM) is a chronic disease, recognized as a high-ranking and daunting health problem of the 21<sup>st</sup> century<sup>[1]</sup>. The rising incidence and prevalence of DM are becoming alarmingly evident irrespective of age groups or gender and occur in the developed and developing countries<sup>[1]</sup>. The International Diabetes Federation alert indicates that if lifestyle and health habits are not drastically and quickly changed, one-half of the Saudis will be diabetic by 2030. Also, as nearly 3 million (18%) Saudi children are overweight or obese, they will be most

vulnerable to acquiring DM<sup>[2]</sup>.

Type 1 diabetes is the presence of antibodies which attack the insulin-producing pancreatic beta cells an indication that type 1 diabetes is an autoimmune disorder. Whereas type 2 diabetes is characterized by insulin resistance and relative insulin deficiency, either or both of which may be present at the time diabetes is diagnosed. Traditionally, anyone exhibiting polyuria, polydipsia, and polyphagia, the classic symptoms of DM, and who also possess a family history of type 1 DM (T1D), obesity, acanthosis nigricans and the absence of both ketosis and diabetes-associated autoantibodies is recognized as a type 2 diabetic (T2D)<sup>[3]</sup>. However, T1D patients are most often thin and may have ketosis and diabetes-linked autoantibodies<sup>[4]</sup>. The DD patients exhibit characteristics of both T1D and T2D, which can be evident either during diagnosis or develop subsequently over time<sup>[5-7]</sup>.

It was in 1991 that the nomenclature "double diabetes" was first given to T1D patients with a family history of T2D, as they were found to more likely be overweight and rarely had sufficient glycemic control, even on high insulin dosages<sup>[8]</sup>. The present classification makes it difficult to describe the type of heterogeneous DM affecting such young patients, whether to categorize them as T2D because of their obesity and insulin resistance, or as T1D due to the presence of auto-antibodies to the  $\beta$  cells<sup>[6]</sup>. Further, DD is quite hard to control, including the micro- and mostly macro-vascular typically T2D-associated complications<sup>[1,6]</sup>.

Although the prevalence and incidence of DD is yet to be clearly defined, however nearly 25% of the T1D children showed obesity or were overweight<sup>[9]</sup>. Also, roughly 35% of children and adolescents with T2D possessed at least one diabetes-related antibody<sup>[9-11]</sup>. The rapid increase in the prevalence of T1D and T2D in the Kingdom caught the interest of the medical world soon after the rapid industrialization resulted in a dramatic spurt in the standard of living and incorporation of "Westernized" habits, including the selection of unhealthy dietary patterns, and reduction in physical activity<sup>[12]</sup>. In Saudi Arabia the prevalence of DM is at an alarming juncture and rising<sup>[1]</sup>. However, no study, to our knowledge, is currently available on the prevalence of DD in Saudi Arabia. Therefore, our objective is to ascertain the prevalence, clinical and biological features of DD among the young Saudi populace.

### MATERIALS AND METHODS

#### Study design, setting and sampling

This is a retrospective descriptive study was conducted among 312 young newly diagnosed diabetic patients (aged 12-20 years) admitted over a 4-year period (January 2009 to December 2013) at Prince Sultan Military Medical City (PSMMC). PSMMC is a 1200-bed, tertiary medical center in Riyadh, Saudi Arabia, with round 40000 annual admissions (950000 active patients files) and 118000 emergency room visits per year from different region of the country. Patients selection of this

**Table 1** Characteristics of the study population (*n* = 312)

Patients characteristics	Yes % ( <i>n</i> )	No % ( <i>n</i> )
Family history of diabetes	57 (178)	43 (134)
Patients with overweight or obese	64 (199)	36 (113)
Diabetic ketoacidosis at presentation	39 (122)	61 (190)
Autoantibodies positivity	52 (162)	48 (150)
Acanthosis nigricans	34 (106)	66 (206)
Family history of auto-immune disease	23 (72)	77 (240)
History of auto-immune disease	18 (56)	82 (256)

study was conducted using eligibility screening.

### Data collection

Family history of DM (first degree), physical body mass index (BMI), acanthosis nigricans, history of auto-immune disease, first degree family history of auto-immune disease (celiac disease, systemic lupus erythematosus, Sjögren's syndrome, thyroid dysfunction (primary hypothyroidism, graves' disease), psoriasis, Crohn's disease, Addison's disease, multiple sclerosis, and myasthenia gravis) and laboratory data for glycosylated hemoglobin (HbA1c), basal C-peptide level and diabetes autoantibody response (anti-GAD, anti-IA2 and anti-ICA) were collected.

### BMI

BMI was calculated by dividing the weight (kg) by the square of height in meters (BMI; kg/m<sup>2</sup>) and BMI z score (adjusted for child age and gender). The z score (standard deviation scores), was figured as per the formula  $(X_i - M_x) / SD$ , where  $X_i$  is the actual measurement,  $M_x$  is the mean value for that age and gender, and SD is the standard deviation corresponding to that age and gender<sup>[13]</sup>.

### Diabetic ketoacidosis

Diabetic ketoacidosis (DKA) is defined as all three of the following must be present: (1) blood glucose level higher than > 250 mg/dL; (2) presence of urine ketones ++ or more; and (3) venous pH level lower than 7.30 and/or serum bicarbonate lower than 15 mEq/L<sup>[14]</sup>.

### HbA1c

HbA1c  $\geq 6.5\%$  makes the diagnosis of DM. The HbA1c test was performed in our laboratory using a standard method (National Glycohemoglobin Standardization Program certified) and standardized to the diabetes control and complications trial assay. In the absence of unequivocal hyperglycemia, results were confirmed by repeat testing<sup>[15]</sup>.

### Statistical analysis

Data analysis was carried out using Microsoft Excel 2010, Microsoft Corporation, Seattle, WA, United States and the Statistical Package for Social Sciences version 20, SPSS Inc., Chicago, IL, United States. In addition to descriptive statistics *t* test and  $\chi^2$  analyses were carried out to compare between DD and others groups. A *P*-value of < 0.05 was considered to be statistically

significant.

## RESULTS

Table 1 lists the characteristics of the population studied. The mean age of patients presenting with DD was  $15.1 \pm 6.4$  years with BMI 26.8 kg/m<sup>2</sup>, and 1:2 sex ratio (male vs female). The results indicated that 57% of the population studied had a family history, 64% showed obesity, 39% had DKA, 52% were positive for autoantibodies, 34% had acanthosis nigricans, 23% possessed a family history of autoimmune disease and 18 had a history of autoimmune disease.

The patients were divided on the basis of the auto-antibody response (Ab+ or Ab-) and C-peptide secretion ( $\beta+$  for fasting level 0.4-2.1 ng/mL and  $\beta-$  if < 0.4 ng/mL) as listed in Figure 1. Depending on the autoantibody response (Ab+ or Ab-) and C-peptide secretion, the patients were segregated into four groups, viz., group1 (type 1a): Ab+  $\beta-$  (21%); group 2 (type 1b): Ab-  $\beta-$  (9%), group 3 (DD): Ab+  $\beta+$  (31%) and group 4 (classic T2D): Ab-  $\beta+$  (39%).

The characteristics of the patients are shown based on the presence or absence of auto-antibodies and the C-peptide secretion are shown in Table 2. More than 25% of the DD population had a family history of diabetes and the mean BMI of the DD population was 26.8 (kg/m<sup>2</sup>). Among the DD population, 28 (38.8%) had family history of auto immune disease, 17 (30.4%) had history of auto immune disease, ninety (31.4%) required insulin at diagnosis and thirty one (21%) required multiple dose of insulin injection during follow-up.

## DISCUSSION

Globally, as the DD population is steadily increasing in number it becomes harder to diagnose and treat because these individuals experience symptoms of both T1D and T2D with the hybrid diabetes<sup>[5,7,16]</sup>. Identifying DD in children and adolescents is crucial as it affects the diagnostic method and choice of treatment. Within the scope of our knowledge, no other study regarding the prevalence of DD in Saudi Arabia is currently available. Therefore, this study was performed to ascertain the prevalence, clinical and biological characteristics of DD among the young Saudi population at PSMC, a tertiary medical center in Saudi Arabia.

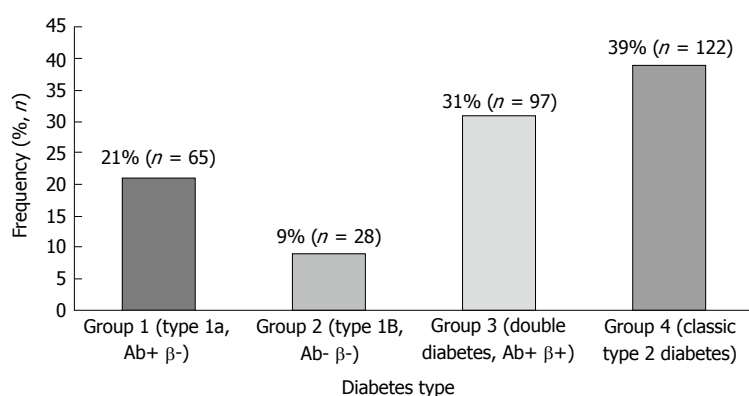
The current study, using the autoantibody response and C-peptide secretion, showed 31% of the population studied with DD and 26.8 mean BMI. However, at present research is limited regarding the incidence and prevalence of DD<sup>[9]</sup>. The results from another study indicated that almost 25% of T1D children are either overweight or obese and have DD<sup>[11]</sup>. This condition usually develops insidiously and initially manifests as a rising requirement for more insulin to control the glucose levels. T2D patients too can be diagnosed with blood tests to identify the specific pancreas-attacking proteins. Some studies also recorded that nearly 35% of children and adolescents with T2D possessed at least one diabetes-related



**Table 2** Characteristics of the patients are shown based on the presence or absence of auto-antibodies and the C-peptide secretion

Auto-antibodies	Ab +		Ab-	
C-peptide secretion	$\beta^-$ (G 1)	$\beta^+$ (double diabetes)	$\beta^-$ (G2)	$\beta^+$
Age of diagnosis	13.16 <sup>a</sup>	15.3	16.6	17.02 <sup>e</sup>
Family history of diabetes	24 (13.5%)	45 (25.3%)	14 (7.9%)	95 (53.4%) <sup>e</sup>
Family history of auto immune disease	32 (44.4%) <sup>a</sup>	28 (38.8%)	5 (6.9%)	7 (9.7%) <sup>e</sup>
History of auto immune disease	29 (51.7%) <sup>a</sup>	17 (30.4%)	2 (3.6%)	8 (14.3%) <sup>e</sup>
DKA at presentation	57 (46.7%) <sup>a</sup>	33 (27%)	5 (4%)	27 (22.1%) <sup>e</sup>
BMI (kg/m <sup>2</sup> )	21 <sup>a</sup>	26.8	24.2	29.6 <sup>e</sup>
HbA1c (%)	10.2 <sup>a</sup>	8.9	10.8	11.7 <sup>e</sup>
Patients requiring insulin at diagnosis	65 (22.6%) <sup>a</sup>	90 (31.4%)	28 (9.8%)	104 (36.2%)
Patients requiring insulin multiple dose injection (follow-up)	65 (44.2%) <sup>a</sup>	31 (21%)	28 (19%) <sup>e</sup>	23 (15.6%) <sup>e</sup>
Patients on metformin only during follow-up	0 <sup>a</sup>	48 (38.1%)	0 <sup>e</sup>	78 (61.9%) <sup>e</sup>
Patients on metformin with insulin during follow-up	4 (6.1%) <sup>a</sup>	27 (41.5%)	2 (3.1%) <sup>e</sup>	32 (49.2%)

Groups compared by Students' *t* test and  $\chi^2$  test: <sup>a</sup>*P* < 0.05, Ab+  $\beta^+$  vs Ab-  $\beta^-$ ; <sup>e</sup>*P* < 0.05, Ab+  $\beta^+$  vs Ab-  $\beta^-$ ; <sup>e</sup>*P* < 0.05, Ab+  $\beta^+$  vs Ab-  $\beta^+$ . DKA: Diabetic ketoacidosis; BMI: Body mass index.

**Figure 1** Patients categorized based on the autoantibody response (Ab+ or Ab-) and C-peptide secretion ( $\beta^+$  for fasting level 0.4-2.1 ng/mL and  $\beta^-$  if < 0.4 ng/mL).

antibody<sup>[10]</sup>. Other studies reported that from among the patients newly diagnosed with diabetes, roughly one out of three children and adolescents suffer from DD<sup>[7]</sup>. DD can be produced by different factors, based on whether the individual initially has T1D or T2D<sup>[7]</sup>. If the patient commences with T1D and begins to gain excess or surplus weight, the individual may begin to be insulin resistant, implying that besides the body being ineffective in secreting insulin due to T1D, the typical insulin injections will no longer be effective as the patient becomes insulin-resistant, which causes the T2D. Such patients then develop DD necessitating medications plus insulin injections for blood sugar level control<sup>[9]</sup>.

Several studies support the fact that DD is often seen in patients with a family history of T2D<sup>[8,17]</sup>, a finding confirmed by the present study where 45% of the study population had a family history of DM. It is noteworthy that nearly 65% of the DD group required insulin, a discovery concurring with earlier reports in the literature<sup>[8,17]</sup>. During follow-up, nearly half the DD patients (46%) were managed solely on metformin (without necessitating insulin therapy).

Significantly, almost 64% of the population in this study was overweight or obese. This higher BMI percentage may possibly be a result of the dramatic rise in the standard of living and the "Westernized" lifestyle habits

adopted in Saudi Arabia. Unhealthy and unwise dietary choices coupled with reduced physical activity have produced this situation<sup>[12,18-20]</sup>. The growing "obesogenic" state that induced insulin resistance could account for the development of islet cell autoimmunity *via* different mechanisms. Therefore, the trend of increasing obesity seems to play a prominent part in the rising incidence and changing phenotype of T1D among adolescents and children<sup>[7]</sup>. Some lifestyle changes and precautions can be incorporated to deter the development of DD for those with and without DM<sup>[12,18,19]</sup>.

### Diagnostic evaluation

Universally applicable clinical diagnostic criteria as well as methods enabling the identification of DD, either at the time of onset of hyperglycemia or during the course of the disease process, must be established. In 2009, Pozzilli *et al*<sup>[9]</sup> introduced the idea of "metabolic load" to define the T2D characteristics and "autoimmune load" to define the T1D features. They revealed that in obese children with hyperglycemia, the presence of a high "metabolic load" and a low "autoimmune load" are indicators of DD<sup>[9]</sup>. Therefore, they presented some biochemical and clinical guidelines to identify DD, as listed: (1) evidence of the clinical characteristics of T2D, dyslipidemia, hypertension and higher BMI with increased

cardiovascular risk, compared with children having classical T1D. Family history for T2D and T1D could be present; (2) a drop in the number of the clinical features of T1D, including polyuria and polydipsia, weight loss, formation of ketoacidosis; in this case insulin therapy is not the first line of treatment, unlike for patients with classical T1D; and (3) the number of autoantibodies to islet cells, although lesser in number and titer when compared with T1D, and sometimes a lower degree of risk linked with the MHC locus compared with T1D patients. Similar to T1D, where insulin resistance and obesity are not the usual characteristics, DD is always distinguished by an obese phenotype, besides the coexistence of  $\beta$  cell autoimmunity<sup>[9]</sup>.

### Limitations

This study includes a few limitations such as the limited number of risk, social, and demographic factors studied and demonstrated in a single center. Further research, preferably conducted on a greater scale, is required to overcome these limitations. However, this study offers pertinent information regarding DD among the young Saudi population.

In conclusion, our findings enable us to arrive at the conclusion that almost one-third of the young Saudi diabetic patients reveal atypical forms of DM (double diabetes) expressing features resulting from both T1D and T2D. Therefore, identification of DD patients becomes crucial as this will give direction for the selection of the most apt diagnostic and therapeutic lines of treatment.

## COMMENTS

### Background

The double diabetes (DD) population is steadily increasing worldwide in number it becomes harder to diagnose and treat because these persons experience symptoms of both type 1 diabetes mellitus (DM) (T1D) and type 2 DM (T2D) with the hybrid diabetes. Classifying DD in children and adolescents is crucial as it affects the diagnostic method and choice of treatment.

### Research frontiers

The present classification makes it difficult to describe the type of heterogeneous DM affecting such young patients, whether to categorize them as T2D because of their obesity and insulin resistance, or as T1D due to the presence of autoantibodies to the  $\beta$  cells. Although the prevalence and incidence of DD is yet to be clearly defined, however nearly 25% of the T1D children showed obesity or were overweight. Also, roughly 35% of children and adolescents with T2D possessed at least one diabetes-related antibody. The rapid increase in the prevalence of T1D and T2D in the Saudi Arabia caught the interest of the medical world soon after the rapid industrialization resulted in a dramatic spurt in the standard of living and incorporation of "Westernized" habits, including the selection of unhealthy dietary patterns, and reduction in physical activity. In Saudi Arabia the prevalence of DM is at an alarming juncture and rising.

### Innovations and breakthroughs

No study, to our knowledge, is currently available on the prevalence of DD in Saudi Arabia. Therefore, the authors ascertained the prevalence, clinical and biological features of DD among the young Saudi populace.

### Applications

The authors' findings enable people to arrive at the conclusion that almost one-third of the young Saudi diabetic patients reveal atypical forms of DM (double

diabetes) expressing features resulting from both T1D and T2D. Therefore, identification of DD patients becomes crucial as this will give direction for the selection of the most apt diagnostic and therapeutic lines of treatment.

### Peer-review

This study provides the prevalence, clinical and biological features of DD in Saudi Arabia. Therefore, the manuscript is good for the readership.

## REFERENCES

- 1 Robert AA, Al Dawish MA, Braham R, Musallam MA, Al Hayek AA, Al Kahtany NH. Type 2 Diabetes Mellitus in Saudi Arabia: Major Challenges and Possible Solutions. *Curr Diabetes Rev* 2016 Jan 26; Epub ahead of print [PMID: 26813972 DOI: 10.2174/1573399812666160126142605]
- 2 Nakamura A, Osonoi T, Terauchi Y. Relationship between urinary sodium excretion and pioglitazone-induced edema. *J Diabetes Investig* 2010; 1: 208-211 [PMID: 24843434 DOI: 10.1111/j.1365-2648.2009.05163.x]
- 3 American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2008; 31 Suppl 1: S55-S60 [PMID: 18165338 DOI: 10.2337/dc08-S055]
- 4 Reinehr T, Schober E, Wiegand S, Thon A, Holl R. Beta-cell autoantibodies in children with type 2 diabetes mellitus: subgroup or misclassification? *Arch Dis Child* 2006; 91: 473-477 [PMID: 16449253 DOI: 10.1136/adc.2005.088229]
- 5 Libman IM, Becker DJ. Coexistence of type 1 and type 2 diabetes mellitus: "double" diabetes? *Pediatr Diabetes* 2003; 4: 110-113 [PMID: 14655267 DOI: 10.1034/j.1399-5448.2003.00012.x]
- 6 Libman IM, Sun K, Foley TP, Becker DJ. Thyroid autoimmunity in children with features of both type 1 and type 2 diabetes. *Pediatr Diabetes* 2008; 9: 266-271 [PMID: 18466208 DOI: 10.1111/j.1399-5448.2008.00400.x]
- 7 Pozzilli P, Guglielmi C, Caprio S, Buzzetti R. Obesity, autoimmunity, and double diabetes in youth. *Diabetes Care* 2011; 34 Suppl 2: S166-S170 [PMID: 21525450 DOI: 10.2337/dc11-s213]
- 8 Teupe B, Bergis K. Epidemiological evidence for "double diabetes". *Lancet* 1991; 337: 361-362 [PMID: 1671252 DOI: 10.1016/0140-6736(91)90988-2]
- 9 Pozzilli P, Guglielmi C. Double diabetes: a mixture of type 1 and type 2 diabetes in youth. *Endocr Dev* 2009; 14: 151-166 [PMID: 19293582 DOI: 10.1159/000207484]
- 10 Hathout EH, Thomas W, El-Shahawy M, Nahab F, Mace JW. Diabetic autoimmune markers in children and adolescents with type 2 diabetes. *Pediatrics* 2001; 107: E102 [PMID: 11389300 DOI: 10.1542/peds.107.6.e102]
- 11 Yki-Järvinen H. Acute and chronic effects of hyperglycaemia on glucose metabolism: implications for the development of new therapies. *Diabet Med* 1997; 14 Suppl 3: S32-S37 [PMID: 9272611 DOI: 10.1007/BF00400200]
- 12 Al Dawish MA, Robert AA, Braham R, Al Hayek AA, Al Saeed A, Ahmed RA, Al Sabaan FS. Diabetes Mellitus in Saudi Arabia: A Review of the Recent Literature. *Curr Diabetes Rev* 2015; 12: 259-368 [PMID: 26206092 DOI: 10.2174/1573399811666150724095130]
- 13 Al-Hayek AA, Robert AA, Abbas HM, Itani MB, Al-Saeed AH, Juhani AE, Al-Goudah HS, Al-Sabaan FS. Assessment of health-related quality of life among adolescents with type 1 diabetes mellitus in Saudi Arabia. *Saudi Med J* 2014; 35: 712-717 [PMID: 25028228]
- 14 Al-Hayek AA, Robert AA, Braham RB, Turki AS, Al-Sabaan FS. Frequency and associated risk factors of recurrent diabetic ketoacidosis among Saudi adolescents with type 1 diabetes mellitus. *Saudi Med J* 2015; 36: 216-220 [PMID: 25719588 DOI: 10.15537/smj.2015.2.10560]
- 15 Al-Rubeaan K. National surveillance for type 1, type 2 diabetes and prediabetes among children and adolescents: a population-based study (SAUDI-DM). *J Epidemiol Community Health* 2015; 69: 1045-1051 [PMID: 26085648 DOI: 10.1136/jech-2015-205710]

- 16 **Pozzilli P**, Buzzetti R. A new expression of diabetes: double diabetes. *Trends Endocrinol Metab* 2007; **18**: 52-57 [PMID: 17208448 DOI: 10.1016/j.tem.2006.12.003]
- 17 **Chillarón JJ**, Flores-Le-Roux JA, Goday A, Benaiges D, Carrera MJ, Puig J, Cano-Pérez JF, Pedro-Botet J. [Metabolic syndrome and type-1 diabetes mellitus: prevalence and associated factors]. *Rev Esp Cardiol* 2010; **63**: 423-429 [PMID: 20334808 DOI: 10.1016/S1885-5857(10)70091-8]
- 18 **Sidawi B**, Al-Hariri MT. The impact of built environment on diabetic patients: the case of Eastern Province, Kingdom of Saudi Arabia. *Glob J Health Sci* 2012; **4**: 126-138 [PMID: 22980349 DOI: 10.5539/gjhs.v4n4p126]
- 19 **Sidawi B**, Alhariri MT, Albaker WI. Creating a healthy built environment for diabetic patients: the case study of the eastern province of the Kingdom of Saudi Arabia. *Glob J Health Sci* 2014; **6**: 136-147 [PMID: 24999135 DOI: 10.5539/gjhs.v6n4p136]
- 20 **Al Hayek AA**, Robert AA, Braham RB, Al Dawish MA. Frequency of Lipohypertrophy and Associated Risk Factors in Young Patients with Type 1 Diabetes: A Cross-Sectional Study. *Diabetes Ther* 2016; **7**: 259-267 [PMID: 26979975]

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## Intermittent energy restriction in type 2 diabetes: A short discussion of medication management

Sharayah Carter, Peter M Clifton, Jennifer B Keogh

Sharayah Carter, Peter M Clifton, Jennifer B Keogh, School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, SA 5000, Australia

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**Correspondence to:** Peter M Clifton, Professor of Nutrition, School of Pharmacy and Medical Sciences, University of South Australia, GPO Box 2471, Adelaide, SA 5000, Australia. [peter.clifton@unisa.edu.au](mailto:peter.clifton@unisa.edu.au)  
Telephone: +61-403-197998

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### Abstract

#### AIM

To discuss type 2 diabetes mellitus (T2DM) medication

changes required during the popular 5:2 intermittent energy restriction (IER) diet.

#### METHODS

A search was conducted in MEDLINE, EMBASE, AMED, CINAHL and Cochrane library for original research articles investigating the use of very low calorie diets (VLCD) in people with T2DM. The search terms used included "VLCD" or "very low energy diet" or "very low energy restriction" or "IER" or "intermittent fasting" or "calorie restriction" or "diabetes mellitus type 2" and "type 2 diabetes". Reference lists of selected articles were also screened for relevant publications. Only research articles written in English, which also included an explanation of medication changes were included. A recent pilot trial using the 5:2 IER method, conducted by our research group, will also be summarized.

#### RESULTS

A total of 8 studies were found that investigated the use of VLCD in T2DM and discussed medication management. Overall these studies indicate that the use of a VLCD for people with T2DM usually require the cessation of medication to prevent hypoglycemia. Therefore, the 5:2 IER method will also require medication changes, but as seen in our pilot trial, may not require total cessation of medication, rather a cessation on the 2 IER days only.

#### CONCLUSION

Guidelines outlined here can be used in the initial stages of a 2-d IER diet, but extensive blood glucose monitoring is still required to make the necessary individual reductions to medications in response to weight loss.

**Key words:** Diabetes mellitus/therapy; Fasting; Caloric restriction; Diabetes complication; Intermittent energy restriction; Obesity; Very low calorie diet; Medication management; Type 2 diabetes mellitus

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**Core tip:** Use of the popular 5:2 intermittent energy restriction diet in people with type 2 diabetes requires careful manipulation of oral hypoglycemic agents and insulin to prevent poor blood glucose control. This short review fills a very important gap in the literature, reviewing necessary medication changes required in severe energy restriction and outlining how these changes may apply during the 5:2 diet by sharing our experiences from our recent 5:2 pilot trial.

Carter S, Clifton PM, Keogh JB. Intermittent energy restriction in type 2 diabetes: A short discussion of medication management. *World J Diabetes* 2016; 7(20): 627-630 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v7/i20/627.htm> DOI: <http://dx.doi.org/10.4239/wjd.v7.i20.627>

## INTRODUCTION

Approximately 80% of people with type 2 diabetes mellitus (T2DM) are overweight or obese<sup>[1]</sup>. Weight loss is known to reduce glycemia and increase insulin sensitivity<sup>[1]</sup> and large amounts of weight loss can lead to remission of T2DM<sup>[2]</sup>. However, weight loss for this population group is often difficult<sup>[3]</sup>, with poor adherence to weight loss programs, suggesting people find continuous energy restriction (CER) difficult to maintain. Recently attention has been given to a new method of weight loss, known as intermittent energy restriction (IER), which in the overweight and obese populations, without diabetes, has shown to be comparable to CER in achieving weight loss<sup>[4,5]</sup>. IER uses short periods (usually 2 d) of severe energy restriction, 400-800 kcal/d, followed by longer periods of habitual diet. There are however, very few studies comparing the effects of IER to daily CER in T2DM. Therefore, we have limited information on how to manage diabetes medications to prevent hypoglycemia, which is likely to occur during the short periods of severe energy restriction. We evaluated continuous very low calorie diet (VLCD) trials to provide a starting point for medication management and to provide guidance to future IER weight loss trials for people with T2DM.

## MATERIALS AND METHODS

A search was conducted in MEDLINE, EMBASE, AMED, CINAHL and Cochrane library for original research articles investigating the use of VLCD in people with T2DM. The search terms used included "VLCD" or "very low energy diet" or "very low energy restriction" or "IER" or "intermittent fasting" or "calorie restriction" or "diabetes mellitus type 2" and "type 2 diabetes". Reference lists of selected articles were also screened for relevant publications. Only research articles written in English, which also included an explanation of medication changes were included.

VLCDs are defined as diets with an energy intake of

< 800 kcal (< 3344 kJ) per day with at least 50 g of high-quality protein, essential fatty acids, daily requirements of vitamins and minerals as well as the addition of approximately 2 cups of non-starchy vegetables to prevent constipation. VLCDs can be given as a complete liquid formula or if food-based diets are used they often include a multivitamin supplement<sup>[6]</sup>.

## RESULTS

Seven trials using continuous VLCD in participants with T2DM were found, and one controlled trial was found using intermittent VLCD (Table 1). In six trials, including the intermittent VLCD trial, all oral hypoglycemic agents (OHA) were discontinued before the start of the trial<sup>[2,7-11]</sup> regardless of the degree of glycemic control. In two trials, medications were reinitiated if blood glucose levels (BGL) were above a pre-determined level<sup>[7,9]</sup>. In one trial, medications, including insulin, were restarted if the mean of two weekly fasting BGL averaged > 13.3 mmol/L for two weeks, dosages were increased thereafter on a case-by-case basis<sup>[7]</sup>. In the second trial, medications were reinitiated at half the original dose if fasting BGLs increased > 13.9 mmol/L<sup>[9]</sup>. In the other four trials, there was no mention of reinitiating medications<sup>[2,8,10,11]</sup>.

In the two remaining trials, diabetic medications and insulin were reduced by 50% at either the commencement of the VLCD treatment<sup>[12]</sup> or in accordance with self-monitored BGLs<sup>[13]</sup>. In one trial, participants measured fasting and postprandial BGLs daily for two days before the start of the VLCD and if the mean result was < 8 and < 10 mmol/L, respectively, diabetic medications were halved<sup>[13]</sup>. Conversely, if levels were > 9 and > 11 mmol/L, respectively, medications were increased<sup>[13]</sup>. Medication changes occurred in the following order; insulin was decreased first followed by sulfonylureas and lastly metformin, and when increasing, medications were increased in reverse order<sup>[13]</sup>. In the second trial, medications were halved at the initiation of the VLCD and reduced further if the fasting weekly average was < 8.4 mmol/L or if participants experienced hypoglycemia (< 3.4 mmol/L) and increased if fasting weekly BGLs averaged > 8.4 mmol/L<sup>[12]</sup>. All changes to dosages occurred on a case-by-case basis in both trials<sup>[12,13]</sup>.

One trial used a VLCD in an overweight population with T2DM on an intermittent basis. The severe energy restriction was used at a frequency of either 1 d or 5 d per week over 20 wk. Oral glycemic agents were discontinued 2 wk before the start of the trial and people with fasting glucose > 16.7 mmol/L were excluded. People using insulin were also excluded from this trial. Medication was reinstated, at half the original dose, if fasting BGLs increased to > 13.9 mmol/L; participants were only required to measure their fasting BGLs levels twice per week.

We recently conducted a 3-mo pilot trial testing the effects of a 2-d IER compared to a CER diet in people with T2DM<sup>[14]</sup>. Our pilot trial demonstrated that 2 d of

Table 1 Summary of trials

Ref.	Design	Duration	Subjects	Aim	Diet groups	Medication protocol
Wing <i>et al</i> <sup>[7]</sup>	Randomized parallel study	50 wk 1-yr follow-up	<i>n</i> = 93 Male/female: 33/60 Mean age: 51.8 ± 9.7 Mean diagnosis (yr): 6.8 ± 6.1	Effects of a weight control program, with and without 2 × 12-wk VLCD restriction	VLCD = 400-500 kcal <i>via</i> liquid or food-based diet from 1-12 wk and from 24-36 wk. LCD was followed at all other times LCD = 1000-1200 kcal	All medications (inc. insulin) were discontinued at the start of the trial. Insulin was discontinued and monitored for 3 d. Dosages of oral medications or insulin were reinstated if the mean of two fasting blood glucose levels averaged > 13.3 mmol/L over a fortnight. Dosages increased on a case-by-case basis
Kelley <i>et al</i> <sup>[8]</sup>	Single arm study	24 wk	<i>n</i> = 7 Male/female: 2/5 Mean age: 59 Mean diagnosis (yr): N/A	Evaluating the efficacy of VLCD treatment in obese T2DM participants	VLCD = 400-800 kcal <i>via</i> liquid and food-based diet	Discontinued all oral glycemic medication 3 wk before commencement on the VLCD
Williams <i>et al</i> <sup>[9]</sup>	Randomized parallel study	20 wk	<i>n</i> = 54 Male/female: 23/31 Mean age: 51.9 ± 7.8 Mean diagnosis (yr): N/A	Evaluating the efficacy of intermittent VLCD restriction on weight loss and glycemic control compared to moderate calorie restriction	VLCD = 400-600 kcal <i>via</i> food-based diet. LDC (1500-1800 kcals) at all other times 2 groups: 1-d: 1 d/wk plus 5 consecutive days in week 2 5-d: 5 d/wk for 15 wk	Discontinued all oral glycemic medication 2 wk before the trial and people with fasting glucose > 16.7 mmol/L were excluded. People using insulin were also excluded. Medications were only reinstated if fasting BGLs (measured twice weekly) increased > 13.9 mmol/L. Restarted medication occurred at half of the original dose
Uusitupa <i>et al</i> <sup>[10]</sup>	Single arm study	12 wk	<i>n</i> = 10 Male/female: 6/4 Mean age: 51 ± 2.2 Diagnosis (yr): Ranged 4-16	Evaluating the effects of weight loss using a VLCD on metabolic control and cardiovascular risk factors in obese participants with T2DM	VLCD = 500-800 kcals <i>via</i> liquid and food-based diet	Discontinued all oral glycemic medications before the start of the trial

VLCD: Very low calorie diet; LCD: Low calorie diet; T2DM: Type 2 diabetes mellitus; BGL: Blood glucose levels.

IER compared to CER achieves similar reductions in HbA1c ( $-0.7\% \pm 0.9\%$ ;  $P < 0.001$ ) and weight loss ( $-5.8 \pm 3.9$  kg;  $P < 0.001$ )<sup>[14]</sup>. In the pilot trial, our protocol was to discontinue OHA likely to cause hypoglycemia (*e.g.*, sulfonylureas) at baseline if HbA1c was < 8%. Medications such as metformin, gliptins, and SGLT2 inhibitors remained unchanged. Participants using insulin were also asked to reduce their dose by 10 units/d if randomized to the CER group or halved on the IER days. If HbA1c was > 8% at baseline OHA remained the same and insulin dose was decreased by 5-10 units on IER days. However, due to low BGLs in some participants we changed the medication protocol in preparation for our 12-mo intervention trial, which is currently ongoing. The new protocol requires discontinuation of sulfonylureas as well as insulin if baseline HbA1c is < 7% for both groups. If HbA1c is > 7% but < 10% then medications are discontinued only on IER days and if HbA1c is > 10% medications remain unchanged. Following this change, there has been a reduction in hypoglycemic events for participants taking insulin on IER days and a reduction in

hyperglycemic events on non-IER days and in the CER group. It is important to note that in addition to changes made based on baseline values, it is also essential to monitor daily BGLs. Each participant requires individual medication changes, especially to insulin units, in response to weight loss.

## DISCUSSION

IER is an alternative method to achieve weight loss, which can be used for the management of T2DM. Due to the severe energy restriction required for IER diets to be effective, management of OHA, as well as insulin, requires constant supervision as well as ongoing blood glucose monitoring by the participant to prevent unwanted hypo- or hyperglycemic events. Medication changes will differ depending on the number of days the intermittent restriction is followed and is likely to only require intervention on these days unless glycemic control is excellent. The treatment method promoted by popular media suggests 2 d of restriction. We tested this

method and we suggest baseline medication changes based on HbA1c, as outlined in the second protocol above, as well as individual changes in response to weight loss. Participants, therefore, need to be willing to monitor their BGLs at least twice daily and report any episodes of hypo- or hyperglycemia, which would indicate the need to further adjust medications.

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## COMMENTS

### Background

Recently attention has been given to a new method of weight loss, known as intermittent energy restriction (IER), which has demonstrated positive results for weight loss in overweight and obese populations.

### Research frontiers

For this new diet method to be used safely in the type 2 diabetes mellitus (T2DM) population, medication management protocol must be established.

### Innovations and breakthroughs

Very low calorie diets used in the treatment of T2DM provide insight, but as seen from the research, medication changes may only be required on the IER treatment days and after weight loss.

### Applications

IER is a successful treatment method for weight loss and glycemic control in T2DM, and with regular blood glucose levels monitoring, medications can be safely adjusted to limit unwanted episodes of hypo-or hyperglycemia.

### Peer-review

The review though very short is written well. The authors state that intermittent energy restriction which requires severe energy restriction needs to be discussed as it is a developing concept.

## REFERENCES

- 1 **Maggio CA**, Pi-Sunyer FX. Obesity and type 2 diabetes. *Endocrinol Metab Clin North Am* 2003; **32**: 805-822, viii [PMID: 14711063 DOI: 10.1016/S0889-8529(03)00071-9]
- 2 **Lim EL**, Hollingsworth KG, Aribisala BS, Chen MJ, Mathers JC, Taylor R. Reversal of type 2 diabetes: normalisation of beta cell function in association with decreased pancreas and liver triacylglycerol. *Diabetologia* 2011; **54**: 2506-2514 [PMID: 21656330 DOI: 10.1007/s00125-011-2204-7]
- 3 **Wing RR**, Marcus MD, Epstein LH, Salata R. Type II diabetic subjects lose less weight than their overweight nondiabetic spouses. *Diabetes Care* 1987; **10**: 563-566 [PMID: 3677974 DOI: 10.2337/diacare.10.5.563]
- 4 **Keogh JB**, Pedersen E, Petersen KS, Clifton PM. Effects of intermittent compared to continuous energy restriction on short-term weight loss and long-term weight loss maintenance. *Clin Obes* 2014; **4**: 150-156 [PMID: 25826770 DOI: 10.1111/cob.12052]
- 5 **Harvie MN**, Pegington M, Mattson MP, Frystyk J, Dillon B, Evans G, Cuzick J, Jebb SA, Martin B, Cutler RG, Son TG, Maudsley S, Carlson OD, Egan JM, Flyvbjerg A, Howell A. The effects of intermittent or continuous energy restriction on weight loss and metabolic disease risk markers: a randomized trial in young overweight women. *Int J Obes (Lond)* 2011; **35**: 714-727 [PMID: 20921964 DOI: 10.1038/ijo.2010.171]
- 6 **Baker S**, Jerums G, Proietto J. Effects and clinical potential of very-low-calorie diets (VLCDs) in type 2 diabetes. *Diabetes Res Clin Pract* 2009; **85**: 235-242 [PMID: 19560834 DOI: 10.1016/j.diabres.2009.06.002]
- 7 **Wing RR**, Blair E, Marcus M, Epstein LH, Harvey J. Year-long weight loss treatment for obese patients with type II diabetes: does including an intermittent very-low-calorie diet improve outcome? *Am J Med* 1994; **97**: 354-362 [PMID: 7942937 DOI: 10.1016/0002-9343(94)90302-6]
- 8 **Kelley DE**, Wing R, Buonocore C, Sturis J, Polonsky K, Fitzsimmons M. Relative effects of calorie restriction and weight loss in noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 1993; **77**: 1287-1293 [PMID: 8077323 DOI: 10.1210/jcem.77.5.8077323]
- 9 **Williams KV**, Mullen ML, Kelley DE, Wing RR. The effect of short periods of caloric restriction on weight loss and glycemic control in type 2 diabetes. *Diabetes Care* 1998; **21**: 2-8 [PMID: 9538962 DOI: 10.2337/diacare.21.1.2]
- 10 **Uusitupa MI**, Laakso M, Sarlund H, Majander H, Takala J, Penttilä I. Effects of a very-low-calorie diet on metabolic control and cardiovascular risk factors in the treatment of obese non-insulin-dependent diabetics. *Am J Clin Nutr* 1990; **51**: 768-773 [PMID: 2333833]
- 11 **Paisey RB**, Frost J, Harvey P, Paisey A, Bower L, Paisey RM, Taylor P, Belka I. Five year results of a prospective very low calorie diet or conventional weight loss programme in type 2 diabetes. *J Hum Nutr Diet* 2002; **15**: 121-127 [PMID: 11972741 DOI: 10.1046/j.1365-277X.2002.00342.x]
- 12 **Collins RW**, Anderson JW. Medication cost savings associated with weight loss for obese non-insulin-dependent diabetic men and women. *Prev Med* 1995; **24**: 369-374 [PMID: 7479627 DOI: 10.1006/pmed.1995.1060]
- 13 **Capstick F**, Brooks BA, Burns CM, Zilkens RR, Steinbeck KS, Yue DK. Very low calorie diet (VLCD): a useful alternative in the treatment of the obese NIDDM patient. *Diabetes Res Clin Pract* 1997; **36**: 105-111 [PMID: 9229194 DOI: 10.1016/S0168-8227(97)00038-7]
- 14 **Carter S**, Clifton PM, Keogh JB. The effects of intermittent compared to continuous energy restriction on glycaemic control in type 2 diabetes; a pragmatic pilot trial. *Diabetes Res Clin Pract* 2016; **122**: 106-112 [PMID: 27833048 DOI: 10.1016/j.diabres.2016.10.010]

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