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EDITORIAL

Short acting insulin analogues in intensive care unit patients

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Abstract

Blood glucose control in intensive care unit (ICU) patients, addressed to actively maintain blood glucose concentration within defined thresholds, is based on two major therapeutic interventions: to supply an adequate calories load and, when necessary, to continuously infuse insulin titrated to patients needs: intensive insulin therapy (IIT). Short acting insulin analogues (SAIA) have been synthesized to improve the chronic treatment of patients with diabetes but, because of the pharmacokinetic characteristics that include shorter onset and off-set, they can be effectively used also in ICU patients and have the potential to be associated with a more limited risk of inducing episodes of iatrogenic hypoglycemia. Medical therapies carry an intrinsic risk for collateral effects; this can be more harmful in patients with unstable clinical conditions like ICU patients. To minimize these risks, the use of short acting drugs in ICU patients have gained a progressively larger room in ICU and now pharmaceutical companies and researchers design drugs dedicated to this subset of medical practice. In this article we report the rationale of using short acting drugs in ICU patients (*i.e.*, sedation and treatment of arterial hypertension) and we also describe SAIA and their therapeutic use in ICU with the potential to minimize iatrogenic hypoglycemia related to IIT. The pharmacodynamic and pharmachokinetic characteristics of SAIA will be also discussed.

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Key words: Insulin analogues; Short acting drugs; Intensive insulin therapy; Glycemia management; Intensive care

Core tip: In this article we report the rationale of using short acting drugs in intensive care unit (ICU) patients (*i.e.*, sedation and treatment of arterial hypertension) and we also describe short acting insulin analogues (SAIA) and their pharmacokinetic (PK) and pharmacodynamic profile. SAIA have been synthesized to improve the chronic treatment of patients with diabetes but, because of the PK characteristics that include shorter onset and offset, they can be effectively used also in ICU patients and have the potential to be associated with a more limited risk of inducing episodes of iatrogenic hypoglycemia.

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INTRODUCTION

Blood glucose control in intensive care unit (ICU) patients, addressed to actively maintain blood glucose concentration (BGC) within defined thresholds, is based on two major therapeutic interventions: to supply an adequate calories load and, when necessary, to continuously infuse insulin titrated to patients needs: intensive insulin therapy (IIT)[1,2]. Among the most relevant risks related to active management of BGC is the induction of iatrogenic hypoglycemia[1-4]. Endogenous insulin is a 51 amino acids protein formed by 2 chains (A and B chains) linked

by disulphide bridges: "A" chain comprises 21 amino acids and has an N-terminal helix linked to an anti-parallel C-terminal helix with a critical role in the tertiary structure; "B" chain comprises 30 amino acids and has a central helical segment where it joins the N- and C-terminal helices of the A chain[5]. Physiologically, insulin is released by the pancreas with a characteristic biphasic profile as response to BGC increase: a rapid phase, due to exocytosis of "ready pool" granules and associated with the release of 5%-10% of the insulin contained in the beta cells, is activated within few minutes after an increase in BGC and terminates rapidly; a slow phase, due to the release of "reserve pool" granules, and lasts longer. Beside BGC driven insulin release, there is also a continuous insulin secretion throughout the day, not associated with meals that accounts for about 50% of the whole daily endogenous insulin secretion[5].

As underlined by several authors and by the pathophysiology of chetoacidosis in diabetic patients and in ICU patients, to supply an adequate calories load is a preliminary step for optimal management of BGC and should be established before insulin infusion is instituted, even in patients with high BGC values[1,2,6].

Currently the standard of care for the treatment of hyperglycemia in ICU patients is to establish intensive insulin therapy by infusing rapid (R) insulin but-and this is among the most important drawback of this therapeutic approach-it induces some additional risk of iatrogenic hypoglycemia[1]. Various strategies have been used to minimize the risk of inducing hypoglycemia when IIT is instituted, these include: to adopt a tighter BGC monitoring protocol, to target a narrower BGC range, to increase the supplied calories load[1,7-10].

In 2001, a large randomized controlled trial in critically ill surgical patients demonstrated that tight glucose control (defined as the restoration and maintenance of BCG at or below 6.1 ± 2.1 mmol/L) by IIT was associated with a decreased mortality and rate of complications[6]. Currently, other authors demonstrated that the incidence of moderate hypoglycemia was significantly increased when target was BGC < 6.7 mmol/L and BGC < 8.3 mmol/L may be a reasonable target for clinical practice[8]. Widening the target-range BGC might reduce the risk of hypoglycemia and hyperglycemia developing, thus limiting neuronal damage[2]. In the subgroup of neurocritical care patients both hypoglycemia and hyperglycemia may cause extended neuronal damage and potentially long-lasting brain injury[1,2]. These patients must therefore undergo strict glycemia monitoring and abnormal blood glucose values should be immediately corrected[1].

In this article we report the rationale of using short acting drugs in ICU patients (*i.e.,* sedation and treatment of arterial hypertension) and we also describe short acting insulin analogues (SAIA) and their therapeutic use in ICU with the potential to minimize iatrogenic hypoglycemia related to IIT. The pharmacodynamic and pharmachokinetic characteristics of SAIA will be discussed.

RATIONALE FOR USING SHORT ACTING DRUGS IN CRITICAL CARE PATIENTS

In pharmaceutical research there is a trend to provide short acting drugs-also called "soft" drugs-to treat critically ill patients and the unstable phase of acute illness and for anesthesia/sedation and perioperative management[11]. The use of short acting vasodilators (*i.e.,* nitroglycerin) in the acute phase of acute myocardial infarction, acute episodes of arterial hypertension in the treatment of the acute phase of heart failure and pulmonary edema is the paradigm of the need for short acting drugs in the treatment of acute illness[12-14]. Recent antihypertensive drugs (as esmolol) and short acting opioids (as remifentanil) are prototypical "soft" drugs designed to fulfill the need for limiting drug-related residual effects when infusion is discontinued[11]. These molecules frequently rely on plasmatic metabolism by non specific bloodstream esterases. A common molecular paradigm to reduce pharmacokinetic (PK) characteristics (including onset and half life) is to modify the parent compound into a "soft" drug by adding an ester linkage, thus, increasing its susceptibility to bloodstream metabolism[11]. In anesthesia new drugs have been developed (midazolam, propofol, desflurane) modifying existing compounds in order to shorten anesthesia induction and awakening times[11,15].

Antihypertensive

Sympathetic stimulation contributes to cerebral hyperemia during emergence from craniotomy. B-blocking drugs may be considered to limit hemodynamic changes of neurosurgical recovery. Esmolol blunted the increase in cerebral blood flow during recovery from neurosurgical anesthesia[16]. Hypertensive emergencies generally require intravenous treatment to achieve a rapid decrease in blood pressure and patients admitted to these care settings may be sicker than patients treated with oral agents. The first choice antihypertensive drug varied by treatment location. In ICU nitroglycerine was by far the most widely used (60%); in the emergency department furosemide was used in 34% of patients and nitroglycerine was used in 27%; perioperatively urapidil was used in 34% of patients and clonidine was used in 28%[12]. While nitroglycerine should be used as an adjunctive therapy, the high rates of use in the European registry for Studying the Treatment of Acute hypertension population likely reflect familiarity with its use, together with its ease of administration, titration and rapid reversibility[12].

Analgesia-sedation

Analgesics and sedatives are commonly prescribed in ICU environment for patient comfort; however, recent studies have shown that these medications can themselves lead to adverse patient outcomes[17]. The use of short acting medications is associated with improved outcomes such as decreased time of mechanical ventilation and ICU length of stay[17]. Using a short-acting opioid

with short context-sensitive half-life in an analgesia based sedation protocol may significantly decrease the duration of mechanical ventilation and the ICU length of stay even though not significantly in long term sedation, while improving the achievement of sedation goals despite a lower requirement for adjunctive hypnotic agents, with no additional costs. The context-sensitive half-life of remifentanil is significantly shorter than those of other opiates. In the remifentanil group, the decreases in need for mechanical ventilation and ICU length of stay were associated with a significant decrease in the use of add-on hypnotics, suggesting that remifentanil was faster adjustable to the required sedation level[18].

Regarding sedation, Clinical Practice Guidelines[19] recommend the use of propofol-rapid onset of sedation (highly lipid soluble and quickly crosses the blood-brain barrier), and rapid offset (quickly redistribution with high hepatic and extrahepatic clearance)-and dexmedetomidine (selective α2-receptor agonist rapidly redistributed into peripheral tissues) over benzodiazepines fot ICU sedation.

Inhaled anesthetics (short acting drugs) may be ideal sedatives for the ICU[20] because of their pulmonary elimination, limited amount of metabolism, bronchodilation and cardioprotective effects[21]. However, inhaled anesthetics are not widely used for sedation in the ICU, since most modern ICU ventilators do not readily accommodate an anesthetic vaporizer. The new anesthetic conserving device, AnaConDa (Sedana Medical™, Sweden) uses a syringe pump to deliver inhaled anesthetic in liquid form into the breathing circuit of a standard ICU ventilator. Belda *et al*[22] adapted a classical PK model to obtain an infusion scheme for the clinical use of the AnaConDa with sevoflurane. Another short acting drug in ICU.

SHORT ACTING INSULIN ANALOGUES

SAIA were developed to improve postprandial glycemic control and to minimize BGC excursions in diabetic patients[23-25]. Due to a PK profile closer to that of endogenous insulin, when physiologically released by the trigger of meals, SAIA have a faster rise in plasma concentration, higher peak concentration and shorter subcutaneous residence time than unmodified human insulin[26]. The clinical use of SAIA is associated with lower postprandial peak BGC as compared with rapid insulin and doesn't increase the incidence of hypoglycemia[23-25].

Currently, 3 SAIA are available for clinical use: lyspro insulin (Humalog®; Eli Lilly, Indianapolis, IN, United States), aspart insulin (Novolog®/NovoRapid®; Novo Nordisk, Bagsvaerd, Denmark) and glulisine insulin (Apidra®; Sanofi, Paris, France).

Lyspro insulin, first SAIA that became available for clinical use in 1996, is characterized by a change in the amino acid sequence of insulin B chain-proline in position 28 and lysine in position 29 are inverted [Lys(B28), Pro(B29)]-that results in a reduced self association[27-29]. These changes result in an insulin molecule with a reduced capacity for self-association[27,28]. Proline at position B28 near the COOH-terminal of the B-chain of human insulin is important for the proper configuration of a p-sheet involving residues B24 through B26. Two insulin molecules align along this surface in an antiparallel orientation to form a nonpolar dimer. At this point, the nonpolar dimer interacts with zinc to form a hexamer, the basis of Regular insulin formulations. The sequence of lysine at B28 and proline at B29 can be found in insulin-like growth factor I (IGF- I) and is thought to be responsible for its lower degree of self-association in comparison to insulin. Accordingly, IGF- I is the model upon which the structure of lyspro is based[27-29]. As a result of these modifications, lyspro exhibits monomeric behavior in solution, binds zinc less avidly, and displays faster pharmacodynamic action than human Regular insulin (Humulin R®). These findings are consistent with the rapid absorption expected from monomeric insulin injected subcutaneously[27,29].

Aspart insulin, second SAIA to achieve regulatory approval in 2000, is characterized by a change in the amino acid sequence of insulin B chain-proline in position 28 is substituted with the charged aspartic acid-this reduces self-association of the molecule, allowing only weak dimeric and hexameric formation and thereby rapid dissociation after subcutaneous injection[27,29,30]. Receptor interaction kinetic studies have shown that aspart insulin behaves essentially like human insulin with regard to both the insulin and IGF- I receptor with a similar potency to that of human insulin[29,30]. Aspart insulin is absorbed twice as fast as regular insulin and reaches a maximum concentration in plasma of approximately twice that of human insulin. Its activity profile is very similar to that of human insulin[29,30].

Glulysin insulin, third SAIA to receive regulatory approval, is characterized by a change in the amino acid sequence of insulin B chain-lysine and glutamic acid are substituted for asparagine and glycine in positions 3 and 29 respectively-it is thought that this latter substitution is predominantly responsible for its PK properties[27,29,31]. Studies indicate that glulisine has a very comparable PK and pharmacodynamic profile to insulin lispro[27,29,31]. Overall, the bioequivalence of glulisine is similar to that of human insulin[27,29,31].

DISCUSSION

In this review article we originally report the use of SAIA in critical care patients. The pharmacodynamic and pharmacokinetic characteristics of SAIA available for clinical use are described and the rationale for using shorter acting insulin is presented.

Altered pharmacology in the intensive care unit

Critically ill patients, not infrequently present alterations of physiological parameters that determine the success/failure of therapeutic interventions as well as the final outcome[32]. Most common and complex syndromes occurring in ICU affect drug absorption, disposition, metabolism and elimination[33]. Pharmacological man-

agement of ICU patients requires consideration of the unique PKs associated with these clinical conditions and the likely occurrence of drug interaction[34]. Rational adjustment in drug choice and dosing contributes to the appropriateness of treatment of those patients[35].

Adverse drug events in intensive care unit

Intensive care medicine provides great benefits to patients with life-threatening acute illness or trauma. These benefits are a consequence of advancements in diagnostic testing, technological interventions and pharmacotherapy. Simultaneously, the complexity and intensity of care required by ICU patients is also associated with greater risks resulting from care[36]. Adverse drug events (ADEs), including adverse reactions and medication errors, are harmful and occur with alarming frequency in critically ill patients[37].

Patients in ICUs may be at especially high risk of an ADE for the following reasons[38,39]: (1) The complexity of diseases; (2) Pathophysiological status characterized by a wide range of changes in organ dysfunction (altering PKs); (3) The high number of medications administered; (4) Administration of complex drug regimens; and (5) Increased length of hospital stay. Hypoglycemia and hyperglycemia are in the 10 top ADE in the ICU[40].

Drug-drug interactions in ICU

Drug-drug interactions (DDIs) in the ICU are associated with longer ICU stays, ADE and end-organ damage[41]. Critically ill patients are at an increased risk of ADE related to DDIs because of the large number of medications that they receive and PK characteristics of the administered medications[42].

The 10 most frequently ocurring DDI in the ICU include insuline/metoprolol (moderate severity rating, β-blockers may enhance the hypoglycemic effects of insulin) and insulin/prednisone (moderate severity rating, corticosteroids may diminish the hypoglycaemic effect of antidiabetic agents)[43].

In this context, medical therapies carry an intrinsic risk for collateral effects; this can be more harmful in patients with unstable clinical conditions like ICU patients[44]. To minimize these risks, the use of short acting drugs in ICU patients have gained a progressively larger room in ICU and now pharmaceutical companies and researchers design drugs dedicated to this subset of medical practice[11]. SAIA have been synthesized to improve the chronic treatment of patients with diabetes but, because of the PK characteristics that include shorter onset and offset, they can be effectively used also in ICU patients and have the potential to be associated with a more limited risk of inducing episodes of iatrogenic hypoglycemia. Clinical studies addressed to assess the dosing profile and the safety of SAIA when used-as intravenous continuous therapy- to accomplish IIT in ICU patients.

REFERENCES

1 **Bilotta F**, Giovannini F, Caramia R, Rosa G. Glycemia management in neurocritical care patients: a review. *J Neurosurg Anesthesiol* 2009; **21**: 2-9 [PMID: 19098617 DOI: 10.1097/ANA.0b013e31818f8a5c]

2 **Bilotta F**, Rosa G. Glycemia management in critical care patients. *World J Diabetes* 2012; **3**: 130-134 [PMID: 22816025 DOI: 10.4239/wjd.v3.i7.130]

3 **Bilotta F**, Caramia R, Paoloni FP, Delfini R, Rosa G. Safety and efficacy of intensive insulin therapy in critical neurosurgical patients. *Anesthesiology* 2009; **110**: 611-619 [PMID: 19237874 DOI: 10.1097/ALN.0b013e318198004b]

4 **Bilotta F**, Rosa G. Glucose management in the neurosurgical patient: are we yet any closer? *Curr Opin Anaesthesiol* 2010; **23**: 539-543 [PMID: 20689410 DOI: 10.1097/ACO.0b013e32833e150a]

5 **Wilcox G**. Insulin and insulin resistance. *Clin Biochem Rev* 2005; **26**: 19-39 [PMID: 16278749]

6 **van den Berghe G**, Wouters P, Weekers F, Verwaest C, Bruyninckx F, Schetz M, Vlasselaers D, Ferdinande P, Lauwers P, Bouillon R. Intensive insulin therapy in critically ill patients. *N Engl J Med* 2001; **345**: 1359-1367 [PMID: 11794168]

7 **Lena D**, Kalfon P, Preiser JC, Ichai C. Glycemic control in the intensive care unit and during the postoperative period. *Anesthesiology* 2011; **114**: 438-444 [PMID: 21245729 DOI: 10.1097/ALN.0b013e3182078843]

8 **Okawa M**, Kunimoto F, Kanamoto M, Narahara H, Hinohara H, Tobe M, Yanagisawa A, Saito S. Effect of different blood glucose target levels on the incidence of hypoglycemia during insulin therapy in the intensive care unit. *J Diabetes* 2013; **5**: 51-56 [PMID: 23035699 DOI: 10.1111/1753-0407.12010]

9 **Heller S**. Reducing hypoglycaemia with insulin analogues. *Int J Obes Relat Metab Disord* 2002; **26** Suppl 3: S31-S36 [PMID: 12174321]

10 **Derde S**, Vanhorebeek I, Ververs EJ, Vanhees I, Darras VM, Van Herck E, Larsson L, Van den Berghe G. Increasing intravenous glucose load in the presence of normoglycemia: effect on outcome and metabolism in critically ill rabbits. *Crit Care Med* 2010; **38**: 602-611 [PMID: 19851097 DOI: 10.1097/CCM.0b013e3181c03f65]

11 **Chitilian HV**, Eckenhoff RG, Raines DE. Anesthetic drug development: Novel drugs and new approaches. *Surg Neurol Int* 2013; **4**: S2-S10 [PMID: 23653886 DOI: 10.4103/2152-7806.109179]

12 **Vuylsteke A**, Vincent JL, de La Garanderie DP, Anderson FA, Emery L, Wyman A, Rushton-Smith S, Gore JM. Characteristics, practice patterns, and outcomes in patients with acute hypertension: European registry for Studying the Treatment of Acute hyperTension (Euro-STAT). *Crit Care* 2011; **15**: R271 [PMID: 22087790 DOI: 10.1186/cc10551]

13 **Flaherty JT**, Reid PR, Kelly DT, Taylor DR, Weisfeldt ML, Pitt B. Intravenous nitroglycerin in acute myocardial infarction. *Circulation* 1975; **51**: 132-139 [PMID: 803231]

14 **Kaltenbach M**, Bussmann WD, Kober G. [Nitroglycerin in the therapy of coronary insufficiency and heart failure]. *Z Kardiol* 1976; **65**: 424-434 [PMID: 820103]

15 **Bilotta F**, Doronzio A, Cuzzone V, Caramia R, Rosa G. Early postoperative cognitive recovery and gas exchange patterns after balanced anesthesia with sevoflurane or desflurane in overweight and obese patients undergoing craniotomy: a prospective randomized trial. *J Neurosurg Anesthesiol* 2009; **21**: 207-213 [PMID: 19542997 DOI: 10.1097/ANA.0b013e3181a19c52]

16 **Bilotta F**, Lam AM, Doronzio A, Cuzzone V, Delfini R, Rosa G. Esmolol blunts postoperative hemodynamic changes after propofol-remifentanil total intravenous fast-track neuroanesthesia for intracranial surgery. *J Clin Anesth* 2008; **20**: 426-430 [PMID: 18929282 DOI: 10.1016/j.jclinane.2008.04.006]

17 **McGrane S**, Pandharipande PP. Sedation in the intensive care unit. *Minerva Anestesiol* 2012; **78**: 369-380 [PMID: 22240616]

18 **Futier E**, Chanques G, Cayot Constantin S, Vernis L, Barres A,

Guerin R, Chartier C, Perbet S, Petit A, Jabaudon M, Bazin JE, Constantin JM. Influence of opioid choice on mechanical ventilation duration and ICU length of stay. *Minerva Anestesiol* 2012; **78**: 46-53 [PMID: 21971434]

19 **Barr J**, Fraser GL, Puntillo K, Ely EW, Gélinas C, Dasta JF, Davidson JE, Devlin JW, Kress JP, Joffe AM, Coursin DB, Herr DL, Tung A, Robinson BR, Fontaine DK, Ramsay MA, Riker RR, Sessler CN, Pun B, Skrobik Y, Jaeschke R. Clinical practice guidelines for the management of pain, agitation, and delirium in adult patients in the intensive care unit. *Crit Care Med* 2013; **41**: 263-306 [PMID: 23269131 DOI: 10.1097/CCM.0b013e3182783b72]

20 **Kong KL**, Bion JF. Sedating patients undergoing mechanical ventilation in the intensive care unit--winds of change? *Br J Anaesth* 2003; **90**: 267-269 [PMID: 12594134]

21 **De Hert SG**, Van der Linden PJ, Cromheecke S, Meeus R, Nelis A, Van Reeth V, ten Broecke PW, De Blier IG, Stockman BA, Rodrigus IE. Cardioprotective properties of sevoflurane in patients undergoing coronary surgery with cardiopulmonary bypass are related to the modalities of its administration. *Anesthesiology* 2004; **101**: 299-310 [PMID: 15277911]

22 **Belda JF**, Soro M, Badenes R, Meiser A, García ML, Aguilar G, Martí FJ. The predictive performance of a pharmacokinetic model for manually adjusted infusion of liquid sevofluorane for use with the Anesthetic-Conserving Device (AnaConDa): a clinical study. *Anesth Analg* 2008; **106**: 1207-1214, table of contents [PMID: 18349194 DOI: 10.1213/ane.0b013e31816782ff]

23 **Lindholm A**, McEwen J, Riis AP. Improved postprandial glycemic control with insulin aspart. A randomized double-blind cross-over trial in type 1 diabetes. *Diabetes Care* 1999; **22**: 801-805 [PMID: 10332685]

24 **Heinemann L**, Hompesch M, Flacke F, Simms P, Pohl R, Albus K, Pfützner A, Steiner S. Reduction of postprandial glycemic excursions in patients with type 1 diabetes: a novel human insulin formulation versus a rapid-acting insulin analog and regular human insulin. *J Diabetes Sci Technol* 2011; **5**: 681-686 [PMID: 21722583]

25 **Luijf YM**, van Bon AC, Hoekstra JB, Devries JH. Premeal injection of rapid-acting insulin reduces postprandial glycemic excursions in type 1 diabetes. *Diabetes Care* 2010; **33**: 2152-2155 [PMID: 20693354 DOI: 10.2337/dc10-0692]

26 **Home PD**. The pharmacokinetics and pharmacodynamics of rapid-acting insulin analogues and their clinical consequences. *Diabetes Obes Metab* 2012; **14**: 780-788 [PMID: 22321739 DOI: 10.1111/j.1463-1326.2012.01580.x]

27 **Howey DC**, Bowsher RR, Brunelle RL, Rowe HM, Santa PF, Downing-Shelton J, Woodworth JR. [Lys(B28), Pro(B29)]-human insulin: effect of injection time on postprandial glycemia. *Clin Pharmacol Ther* 1995; **58**: 459-469 [PMID: 7586939]

28 **DiMarchi RD**, Mayer JP, Fan L, Brems DN, Frank BH, Green LK, Hoffman JA, Howey DC, Long HB, Shaw WN, Shields JE, Slieker LJ, Su KSE, Sundell KL, Chance RE. Synthesis of a fast-acting insulin based on structural homology with insulin-like growth factor 1. In Peptides: chemistry and biology, proceedings of the twelfth american peptide symposium. Smith JA, Rivier JE, Eds. Leiden, Netherlands, ESCOM, 1992: 26-28

29 **Sheldon B**, Russell-Jones D, Wright J. Insulin analogues: an example of applied medical science. *Diabetes Obes Metab* 2009; **11**: 5-19 [PMID: 19120431 DOI: 10.1111/j.1463-1326.2008.01015.x]

30 **Mudaliar SR**, Lindberg FA, Joyce M, Beerdsen P, Strange P, Lin A, Henry RR. Insulin aspart (B28 asp-insulin): a fast-acting analog of human insulin: absorption kinetics and action profile compared with regular human insulin in healthy nondiabetic subjects. *Diabetes Care* 1999; **22**: 1501-1506 [PMID: 10480516]

31 **Becker RH**, Frick AD, Burger F, Scholtz H, Potgieter JH. A comparison of the steady-state pharmacokinetics and pharmacodynamics of a novel rapid-acting insulin analog, insulin glulisine, and regular human insulin in healthy volunteers using the euglycemic clamp technique. *Exp Clin Endocrinol Diabetes* 2005; **113**: 292-297 [PMID: 15926116]

32 **Rivera-Fernández R**, Nap R, Vázquez-Mata G, Reis Miranda D. Analysis of physiologic alterations in intensive care unit patients and their relationship with mortality. *J Crit Care* 2007; **22**: 120-128 [PMID: 17548023]

33 **Boucher BA**, Wood GC, Swanson JM. Pharmacokinetic changes in critical illness. *Crit Care Clin* 2006; **22**: 255-271, vi [PMID: 16677999]

34 **Roberts JA**, Lipman J. Antibacterial dosing in intensive care: pharmacokinetics, degree of disease and pharmacodynamics of sepsis. *Clin Pharmacokinet* 2006; **45**: 755-773 [PMID: 16884316]

35 **Zagli G**, Tarantini F, Bonizzoli M, Di Filippo A, Peris A, De Gaudio AR, Geppetti P. Altered pharmacology in the Intensive Care Unit patient. *Fundam Clin Pharmacol* 2008; **22**: 493-501 [PMID: 18684127 DOI: 10.1111/j.1472-8206.2008.00623.x]

36 **George EL**, Henneman EA, Tasota FJ. Nursing implications for prevention of adverse drug events in the intensive care unit. *Crit Care Med* 2010; **38**: S136-S144 [PMID: 20502167 DOI: 10.1097/CCM.0b013e3181de0b23]

37 **Kane-Gill S**, Rea RS, Verrico MM, Weber RJ. Adverse-drug-event rates for high-cost and high-use drugs in the intensive care unit. *Am J Health Syst Pharm* 2006; **63**: 1876-1881 [PMID: 16990635]

38 **Kane-Gill SL**, Jacobi J, Rothschild JM. Adverse drug events in intensive care units: risk factors, impact, and the role of team care. *Crit Care Med* 2010; **38**: S83-S89 [PMID: 20502179 DOI: 10.1097/CCM.0b013e3181dd8364]

39 **Joshua L**, Devi P, Guido S. Adverse drug reactions in medical intensive care unit of a tertiary care hospital. *Pharmacoepidemiol Drug Saf* 2009; **18**: 639-645 [PMID: 19421963 DOI: 10.1002/pds.1761]

40 **Reis AM**, Cassiani SH. Adverse drug events in an intensive care unit of a university hospital. *Eur J Clin Pharmacol* 2011; **67**: 625-632 [PMID: 21246350]

41 **Moura C**, Prado N, Acurcio F. Potential drug-drug interactions associated with prolonged stays in the intensive care unit: a retrospective cohort study. *Clin Drug Investig* 2011; **31**: 309-316 [PMID: 21344954 DOI: 10.2165/11586200-000000000-00000]

42 **Reis AM**, Cassiani SH. Prevalence of potential drug interactions in patients in an intensive care unit of a university hospital in Brazil. *Clinics* (Sao Paulo) 2011; **66**: 9-15 [PMID: 21437429]

43 **Smithburger PL**, Kane-Gill SL, Seybert AL. Drug-drug interactions in the medical intensive care unit: an assessment of frequency, severity and the medications involved. *Int J Pharm Pract* 2012; **20**: 402-408 [PMID: 23134100 DOI: 10.1111/j.2042-7174.2012.00221.x]

44 **Van den Berghe G**. What's new in glucose control in the ICU? *Intensive Care Med* 2013; **39**: 823-825 [PMID: 23460140 DOI: 10.1007/s00134-013-2874-3]

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TOPIC HIGHLIGHT

WJD 5th Anniversary Special Issues (1): Insulin

Adipose stem cell-based regenerative medicine for reversal of diabetic hyperglycemia

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Abstract

Diabetes mellitus (diabetes) is a devastating disease that affects millions of people globally and causes a myriad of complications that lead to both patient morbidity and mortality. Currently available therapies, including insulin injection and beta cell replacement through either pancreas or pancreatic islet transplantation, are limited by the availability of organs. Stem cells provide an alternative treatment option for beta cell replacement through selective differentiation of stem cells into cells that recognize glucose and produce and secrete insulin. Embryonic stem cells, albeit pluripotent, face a number of challenges, including ethical and political concerns and potential teratoma formation. Adipose tissue represents an alternative source of multipotent mesenchymal stem cells, which can be obtained using a relatively simple, non-invasive, and inexpensive method. Similarly to other adult mesenchymal stem cells, adipose-derived stem cells (ADSCs) are capable of differentiating into insulin-producing cells. They are also capable of vasculogenesis and angiogenesis, which facilitate engraftment of donor pancreatic islets when co-transplanted. Additionally, anti-inflammatory and immunomodulatory effects of ADSCs can protect donor islets during the early phase of transplantation and subsequently improve engraftment of donor islets into the recipient organ. Although ADSC-therapy is still in its infancy, the potential benefits of ADSCs are far reaching.

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Key words: Diabetes mellitus; Diabetes; Insulin; Stem cells; Adipose; Pancreas; Beta-cells; Differentiation

Core tip: Adipose-derived stem cells (ADSCs) can provide a promising cell therapy for treatment of diabetes and associated complications. ADSCs' multipotency allows differentiation into insulin-producing β-cells. Anti-inflammatory and immunomodulatory capabilities of ADSCs can facilitate enhanced engraftment of transplanted donor islets. Although many challenges lie ahead for ADSC-based cell therapies are used clinically to treat diabetic hyperglycemia, ADSCs represent a novel treatment option to many diabetic patients worldwide.

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INTRODUCTION

Diabetes mellitus (diabetes) is a chronic disease, affecting over 347 million people globally[1-8]. Due to diets with high fat and high sugar accompanied by sedentary lifestyles, the global epidemic of diabetes is expected to rise. Furthermore, the economic burden imposed by diabetes and its complications easily exceeds $100 billion annually[9].

The most common treatment for type 1 and some type 2 diabetes is insulin therapy. Intensive insulin treat-

ment can maintain normoglycemia, and control acute hypoglycemia as well as long-term complications[10,11], however, fails to achieve normal hemoglobin A1c levels. Advancements in commercial glucose monitors, insulin formulation, and insulin pumps are also providing improved control of diabetic symptoms[10,12]. However, even with widely available insulin therapy, the life expectancy of diabetic patients is approximately 12 years shorter on average than that of non-diabetic individuals[9,13]. Additionally, those with child-onset type 1 diabetes have a significantly increased risk of retinopathy, nephropathy, neuropathy, and various cardio-, cerebro- and peripheral vascular diseases[5,6,9,10,14-21].

More definitive treatment options for type 1 diabetes, which is characterized by autoimmune destruction of insulin-producing β-cells in pancreatic islets of Langerhans, are pancreas or pancreatic islet transplantation[22-26]. Over a century ago, pancreas extracts were the first transplants tested in diabetic patients[27]. Modern-day pancreas and pancreatic islet transplantations are relatively effective in normalizing fasting and postprandial blood glucose levels, hemoglobin A1c levels as well as restoring insulin and C-peptide production[9]. However, the severe shortage of available donors limit the widespread adoption of this form of therapy[10,28], and thus, appear to only benefit less than 0.5% of type 1 diabetics[28]. Additionally, life-long requirement of immunosuppression and adverse effects caused by immunosuppressants, such as nephrotoxicity, hypertension, and hypersensitivity to infection, often leads to patient non-compliance[10,28,29]. Lastly, reoccurring autoimmunity against pancreatic β-cells continues to be a major challenge associated with transplantation therapies[9].

Recent advancements in stem cell isolation and differentiation methodologies have resulted in production of cell lines with the capability to synthesize, package, and subsequently secrete insulin in response to glucose. Albeit pluripotent, embryonic stem (ES) cell differentiation often leads to the development of multiple cell lineages, resulting in a mixed population of cells along with target cells[9]. Definitive endodermal markers are also absent in ES cells, and undifferentiated teratogenic ES cells may pose serious risks as well[9,28]. Due to ethical and legal concerns and risks of teratoma formation, embryonic stem cells face austere challenges in becoming a clinically viable solution although cellular isolation device may provide a method to implant embryonic stem cells with insulin producing capabilities[30].

Multipotent progenitor cells are now known to be localized in many different organs[31]. Although multipotent, adult stem cells provide a relatively reliable source of mesenchymal stem cells for cell-based therapies. Recently, adult stem cells from bone marrow, umbilical cord blood, pancreatic duct, periosteum, and adipose tissue have shown a capacity to differentiate into insulin-producing cells[32-43].

Among the many tissue sources for adult stem cells, adipose tissue is particularly attractive based on its stem cell abundance and ease of tissue procurement through a minimally invasive and relatively inexpensive procedure[44-48]. Mesenchymal stem cells from bone marrow and adipose tissue share similar cell populations, along with cell characteristics[49-51]. Adipose tissue has also been reported to contain a significantly greater number of mesenchymal stem cells than bone marrow per unit weight[6,52-54]. In this review, adipose-derived stem cells will be specifically examined for their utility in developing treatments for diabetes and diabetic complications.

Direct differentiation into pancreatic hormone producing cells

Kodama *et al*[55] proposed four mechanisms of pancreatic regeneration: (1) replication of mature β-cells; (2) differentiation of stem cells; (3) cell fusion; and (4) transdifferentiation of one stem cell type to another. Most studies on cell-based therapies focus on direct differentiation of stem cells into insulin-producing β-cells.

Mesenchymal stem cells derived from adipose tissue exhibit unique characteristics well suited for transdifferentiation into a pancreatic endocrine lineage, which is of the endodermal origin. Freshly isolated adipose-derived stem cells (ADSCs) also expressed stem cell factor (SCF) and its receptor (c-kit)[44,56], but not ABCG2, nestin, Thy-1, and Isl-1. Lin *et al*[6] reported that ADSCs constitutively expressed glucagon and NeuroD as well as insulin. The proliferative ADSCs, on the other hand, expressed the transcription factor Isl-1 and Pax-6, which are critical transcription factors required for β cell development[44,56], as a previous study showed that formation of insulin- and glucagon-positive cells were found inhibited during development of Isl-1 knock-out mice[57]. Therefore, the intrinsic expression of Isl-1 in ADSCs provides a considerable advantage for generating insulin-producing cells. Proliferative ADSCs also express stem cell markers nestin, ABCG2, SCF, and Thy-1. Nestin was originally thought to be a neural stem/progenitor cell marker but was recently reported to be a multipotent pancreatic stem cell marker as well, detected within pancreatic islets[16,58]. ABCG2 has also shown to be associated with pancreatic islet-derived precursor cells and neural stem cells[10,59]. Kojima *et al*[60] demonstrated that extrapancreatic insulin-producing cells, which were positive for proinsulin and insulin, were present in the adipose tissue of streptozotocin-induced diabetic rodents. Based on these intrinsic characteristics, ADSCs can serve as a promising source of pancreatic hormone-producing cells following differentiation.

Derivation of insulin producing cells from stem cells is made possible through the understanding of key steps during embryonic development and the coordinated activation of intracellular transcription factors. Similar to embryonic stem cells[61-65], derivation of insulin-producing cells from ADSC is executed through a progressive multistage differentiation protocol: starting from definitive endoderm into pancreatic endoderm and finally into pancreatic hormone-expressing cell[2,44,56,66-68]. Outlines the culture conditions used by various groups to stimulate ADSCs into an insulin-producing cell lineage.

All of the differentiated cell populations reported

were stained positively for dithizone, indicating the presence of endogenous insulin. Furthermore, these stem cell-derived insulin producing cells exhibited abundant expression of Pdx-1, C-peptide, insulin, glucagon, somatostain, pancreatic polypeptide, and Glut-2[2,44,56]. Enhanced expression of Isl-1, Pax-4, Ngn-3, Ipf-1, Pax-6, Nkx-2.2, Nkx-6.1, FoxA2, GLP-1 receptor, and glucokinase was also confirmed in differentiated cells, implicating pancreatic lineage[2,16,44,56,69]. Interestingly, transcription of leptin and adiponectin was also well maintained in differentiated cells, still demonstrating adipose tissue characteristics. Additionally, expression of visfatin, which activates insulin receptors and has a blood glucose lowering effect similar to insulin, was significantly upregulated following differentiation into an insulin producing phenotype[44].

Following transplantation of human ADSC-derived insulin producing cells into streptozotocin-induced diabetic mice, a significant level of human C-peptide was detected in subjects, demonstrating successful insulin production *in vivo*. Although these differentiated cells demonstrated a capacity to lower blood glucose levels, the insulin secretion level compared to mature pancreatic islets was significantly lower, and they failed to restore normoglycemia in STZ-induced diabetic mice[6,44,67].

The ability of ADSCs to differentiate into insulin-producing cells akin to mature native pancreatic cells also remains under question. Dor *et al*[70] used a genetic lineage tracing method to determine whether pancreatic stem cells contribute to pancreatic β-cell replenishment during adult life. In this study, they demonstrated that terminally differentiated mature β-cells maintain their proliferative capacity and serve as a major source of new β-cells in mice, contrary to previously reported studies[71-74]. Although this study directly rejected pluripotent adult stem cells' role in replacing β-cells *in vivo* following partial pancreatectomy, it does not directly refute the utility of insulin-producing cells, differentiated from adult stem cells *in vitro*, as a potential new treatment option for diabetics as demonstrated by a number of studies previously reported[71-74].

Engraftment of transplanted islets

Success of pancreatic islet transplantation depends on successful engraftment into the recipient liver where donor islets are transfused through the hepatic portal vein. However, apoptosis, inflammation and ischemia frequently interfere with successful engraftment[75], and therefore two or more pancreata are frequently required to procure sufficient numbers of islets for each transplant. This is a major limitation to the widespread use of this therapy, considering the acute shortage of donor organs. Due to unavoidable destruction of native islet structures, including intraislet vasculature, during isolation, islet engraftment could take up to several weeks[76,77]. Further deterioration of islets and β-cell death can occur due to ischemia and inflammation, ultimately leading to graft failure[78,79]. A mean to improve engraftment of transplanted islets will lead to a reduction of the required number of pancreata and more positive clinical outcomes.

Adipose-derived stem cells have been reported to possess inherent regenerative angiogenic potential and anti-apoptoic capability through their secretion of trophic factors[80-82]. ADSCs also have anti-inflammatory and immunomodulatory properties, including suppression of T-cell proliferation[82-88]. Therefore, ADSCs can potentially allow improved engraftment of transplanted islets with enhanced vascularization and suppression of inflammation.

Ohmura *et al*[79] tested hybrid islet transplantation by co-transplanting allogeneic mouse pancreatic islets along with autologous ADSC under the kidney capsule of recipient mice and demonstrated that autologous murine ADSCs were able to significantly prolong allogeneic islet survival and achieve normoglycemia for up to 14 d. Allogeneic islets alone could not survive under the kidney capsule for longer than 2 d, and normoglycemia was never achieved. The islets following hybrid transplantation showed well-preserved islet architecture and were surrounded by endothelial cells compared to islet grafts transplanted without ADSCs, suggesting vascularization had been improved. Infiltration by $CD4^+$/$CD8^+$ T cells and $CD68^+$ macrophages were also markedly reduced, suggesting successful anti-inflammation and immunomodulation by ADSCs and prolonged graft islet retention when ADSCs were co-transplanted with donor islets[79]. Although it is still uncertain whether this hybrid transplantation method will work in a clinical model, which utilizes the hepatic portal vein route for islet transplantation rather than the kidney capsule, the potentially enormous benefits of ADSCs in islet engraftment is clearly promising.

Veriter *et al*[89] also showed the utility of ADSCs by co-encapsulating xenogeneic porcine islets with autologous primate ADSCs in semipermeable capsules and transplanting them in primates. Compared to islets encapsulated alone, improved oxygenation, graft survival and function, and glycated hemoglobin correction, as well as greater vasculogenesis were observed in co-encapsulated implants, consequently reducing the cellular stress immediately following transplantation[89].

It is widely accepted that a significantly large number of pancreatic islets are lost during the first 10-14 d following infusion into human liver through the portal vein[90], even in the presence of immunosuppression. Furthermore, 60% of transplanted islets were reported to die during this period even in syngeneic animal models[91]. An ability to prevent such early death immediately following transplantation, as demonstrated by Ohmura *et al*[79], Veriter *et al*[89] and Cavallari *et al*[92], using ADSCs, may prove to be enormously beneficial to the successful engraftment of transplanted islets.

Challenges and opportunities for ADSCs in diabetes

Several uncertain factors in stem cell-based cell therapy for diabetes still remain: (1) the absence of gold-standard,

reproducible differentiation protocol for generating insulin-producing cells from adult stem cells; (2) an exact dosage of stem cell-derived β-cells to reverse diabetic conditions and feasibility of producing such dosage *in vitro*; (3) proliferative capacity and maintenance of differentiated insulin-producing cells; (4) sensitivity to counter-regulatory hormones; (5) potential adverse effects of undifferentiated adult stem cells; and (6) potential *in vivo* migration of differentiated cells following implantation[8,15]. Consensus of investigators on the criteria for transdifferentiation and plasticity to avoid confusion with cell fusion, contaminating stem cell populations, and to prevent over interpretation of the data, is necessary[8,93-95].

A major challenge also lies in imitating the physiological mechanism of insulin secretion. Insulin secretion occurs through complex regulatory systems, involving multiple hormonal feedback mechanisms and neurological stimulation, within the islet of Langerhans. For instance, insulin secretion by β-cells can inhibit glucagon secretion by α-cells[96]. Somatostatin secreted by δ-cells also regulates insulin secretion by β-cell[97]. In order to mimic normal or near normal metabolic control, differentiated cells must be able to interact with existing pancreatic endocrine cells. Another mechanism of controlling insulin release is through the secretion of incretin hormones, including glucose-dependent insulinotropic peptide and glucagon-like peptide 1[10,98-101]. These intestinal tract signaling hormones have shown to be responsible for up to 70% of glucose-induced postprandial insulin secretion[99,100]. An ability to respond to these signals is also a critical characteristic that stem cell-derived β-cells need to possess in order to closely mimic physiological processes. Lastly, insulin secretion is a pulsatile rather than a constant release, and such pulsatility may be significant in its action[102]. Stem cells differentiated into a pancreatic lineage that simply produces insulin, even in a glucose-responsive manner, without capability to accommodate these complex interactions, will unavoidably fail to reverse diabetic conditions.

The general architecture of natural pancreatic islets also poses another challenge for the efficacy of differentiated insulin-producing cells. Individual islets are highly vascularized and innervated. The endothelial cells comprising the microvasculatures of pancreatic islets of Langerhans may even be glucose responsive[10,103]. Stem cell-derived islet-like structures thus far have not shown to contain any intrinsic vascularity within them when derived *in vitro*, and therefore rely on the circulation external to the cell aggregates. The distance between β-cells and capillaries can potentially affect the kinetics of insulin release, and non-physiological integration of islet-like structures to circulation may in turn affect the engraftment, survival, and efficacy of implants[104]. Insulin release by β-cells is affected not only by increased blood glucose level but also by nervous control (cephalic phase) mostly through cholinergic neurons during meal ingestion[10,105]. Even with whole organ or pancreatic islet transplantation, complete restoration of the cephalic phase of insulin secretion will fail due to a lack of innervation[106,107]. These structural challenges are critical to overcome for stem cell-derived β-cells or islets to be clinically viable in the future.

Nearly all of the insulin-producing cells derived from adult stem cells co-express glucagon, somatostatin, pancreatic polypeptide along with insulin, all of which are characteristic of immature pancreatic islets of Langerhans. This suggests an incomplete differentiation of stem cells, and could be one of the main reasons why these cells were unable to achieve normoglycemia in diabetic animals. Further differentiation and maturation are required to achieve a more mature substitute capable of functioning similarly to a normal pancreas. However, others also argue that terminally differentiated mature β-cells might not be required for treatment of diabetes. Konno *et al*[108] and Kajiyama *et al*[109] reported that transplantation of adipose-derived stem cells overexpressing Pdx-1 ameliorated hyperglycemia and improved survival rate. Furthermore, ecto-pancreatic transplantation enabled normalization of hemoglobin A1c levels and subsequently attenuated or partially reversed nerve and kidney damages caused by diabetes[10,110,111]. Achieving normal hemoglobin A1c levels may also prove to be critical for future stem cell-based therapies.

Diabetic conditions present a uniquely detrimental environment to various cell types. The proliferative capability of mesenchymal stem cells isolated from adipose tissue of streptozotocin-induced type 1 and 2 diabetic rats was reported to be compromised[112]. When ADSCs were exposed to high glucose concentration *in vitro* prior to implantation into a hindlimb ischemia model, their proliferative capacity and ability to reverse hindlimb ischemia were significantly and irreversibly reduced, compared to ADSCs cultured at a normal glucose concentration[112]. In type 1 diabetic patients, however, autoimmunity did not seem to fundamentally influence the regenerative capability of islets and their progenitor cells[34,113]. Hess *et al*[114] demonstrated that bone marrow derived stem cells initiated pancreatic regeneration and reversed hyperglycemia by stimulating proliferation of the recipient's innate pancreatic progenitor cells and β-cells. It is highly possible the same mechanism can be utilized for ADSCs, and therefore, warrants further investigation as well. Improving the relative regenerative capacity of pancreatic islets using ADSCs would potentially benefit diabetic patients.

Transplantation of islet-like cells or pancreas-like tissues generated from stem cells *in vitro* may be accompanied by graft rejection, graft hypertrophy with subsequent chronic hypoglycemia, and potentially malignant transformation. The intrinsic immunomodulatory capabilities of ADSCs have shown to enhance engraftment of multiple types of tissues when co-transplanted[115-117]. Vanikar *et al*[115] reported that transfusion of ADSCs may reduce the need of immunosuppression during renal transplantations. The ability to reduce the required dosage of immunosuppressants would subsequently minimize complications caused by these agents and improve the clinical

outcome of islet transplantation.

Approximately 90% of people with diabetes are suffering from type 2 diabetes. However, only a few cases of stem cell-based research were performed recently[118-122] to develop a therapeutic option for type 2 diabetes, as type 1 diabetes has stood as the forefront. Deriving insulin-secreting β-cells from stem cells for treatment of type 1 diabetes seems relatively straightforward compared to developing an alternative treatment option for type 2 diabetes. Further research on the complex disease mechanisms of type 2 diabetes in association with the potential utility of stem cells may improve the quality of life for hundreds of millions patients.

CONCLUSION

It is now undeniable that the utility of ADSCs in the treatment of diabetes is extremely promising. The abundance of available source tissue, high frequency and multipotency of adipose-derived mesenchymal stem cells, its trophic and regenerative capabilities, all serve as valuable solutions to the ever-increasing diabetic population and associated health crises observed around the world. Understanding of ADSCs and the development of ADSC-based treatments for diabetes are still considered to be in their infancy, and numerous challenges and opportunities still lie ahead. The exact mechanism of generating insulin-producing cells from ADSCs as well as further maturation of those cells into functional pancreatic islets still needs to be further explored. Sustainability of differentiated insulin-producing cells is still under investigation. Autoimmune attack on β-cells, which is a fundamental disease mechanism of type 1 diabetes, has not been completely resolved and can make any future cell-based therapy unfeasible.

Current therapies for diabetes ranging from insulin injection to pancreatic islet transplantation are not truly the best options for patients. Stem cells that are theoretically limitless in numbers and multipotent will provide hopes and viable therapies for millions of diabetic patients in the future. However, if all stem cell-based therapies only eliminate the need for glucose monitoring and insulin injection for convenience and modestly improve diabetic symptoms, it would not justify the adoption of these therapies in the future. Therefore, stem cell-based therapies must be able to provide fundamentally improved multifaceted metabolic controls and concomitantly improve long-term prognosis in diabetic patients to be widely accepted as a clinically viable therapy.

REFERENCES

1 **Danaei G**, Finucane MM, Lu Y, Singh GM, Cowan MJ, Paciorek CJ, Lin JK, Farzadfar F, Khang YH, Stevens GA, Rao M, Ali MK, Riley LM, Robinson CA, Ezzati M. National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2·7 million participants. *Lancet* 2011; **378**: 31-40 [PMID: 21705069 DOI: 10.1016/S0140-6736(11)60679-X]

2 **Dhanasekaran M**, Indumathi S, Harikrishnan R, Mishra R, Lissa RP, Rajkumar JS, Sudarsanam D. Human omentum fat-derived mesenchymal stem cells transdifferentiates into pancreatic islet-like cluster. *Cell Biochem Funct* 2013; **31**: 612-619 [PMID: 23315589 DOI: 10.1002/cbf.2948]

3 **Mathers CD**, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med* 2006; **3**: e442 [PMID: 17132052]

4 **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]

5 **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]

6 **Lin G**, Wang G, Liu G, Yang LJ, Chang LJ, Lue TF, Lin CS. Treatment of type 1 diabetes with adipose tissue-derived stem cells expressing pancreatic duodenal homeobox 1. *Stem Cells Dev* 2009; **18**: 1399-1406 [PMID: 19245309 DOI: 10.1089/scd.2009.0010]

7 **Zimmet P**, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. *Nature* 2001; **414**: 782-787 [PMID: 11742409]

8 **Ansari MJ**, Fiorina P, Dada S, Guleria I, Ueno T, Yuan X, Trikudanathan S, Smith RN, Freeman G, Sayegh MH. Role of ICOS pathway in autoimmune and alloimmune responses in NOD mice. *Clin Immunol* 2008; **126**: 140-147 [PMID: 17889619]

9 **Ramiya V**, Schatz D. Islet replacement vs. regeneration: hope or hype? *Pediatr Diabetes* 2004; **5** Suppl 2: 45-56 [PMID: 15601374]

10 **Lechner A**. Stem cells and regenerative medicine for the treatment of type 1 diabetes: the challenges lying ahead. *Pediatr Diabetes* 2004; **5** Suppl 2: 88-93 [PMID: 15601379]

11 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]

12 **Steil GM**, Panteleon AE, Rebrin K. Closed-loop insulin delivery-the path to physiological glucose control. *Adv Drug Deliv Rev* 2004; **56**: 125-144 [PMID: 14741112]

13 **Manuel DG**, Schultz SE. Using linked data to calculate summary measures of population health: Health-adjusted life expectancy of people with Diabetes Mellitus. *Popul Health Metr* 2004; **2**: 4 [PMID: 15038828]

14 **Wei AH**, Wang WJ, Mu XP, Li HM, Yan WQ. Enhanced differentiation of human adipose tissue-derived stromal cells into insulin-producing cells with glucagon-like peptide-1. *Exp Clin Endocrinol Diabetes* 2012; **120**: 28-34 [PMID: 21915820 DOI: 10.1055/s-0031-1280807]

15 **Harvey JN**, Allagoa B. The long-term renal and retinal outcome of childhood-onset Type 1 diabetes. *Diabet Med* 2004; **21**: 26-31 [PMID: 14706050]

16 **Zulewski H**. Stem cells with potential to generate insulin-producing cells in man. *Swiss Med Wkly* 2007; **137** Suppl 155: 60S-67S [PMID: 17874504]

17 **Venturini M**, Fiorina P, Maffi P, Losio C, Vergani A, Secchi A, Del Maschio A. Early increase of retinal arterial and venous blood flow velocities at color Doppler imaging in brittle type 1 diabetes after islet transplant alone. *Transplantation* 2006; **81**: 1274-1277 [PMID: 16699454]

18 **Maffi P**, Bertuzzi F, De Taddeo F, Magistretti P, Nano R, Fiorina P, Caumo A, Pozzi P, Socci C, Venturini M, del Maschio A, Secchi A. Kidney function after islet transplant alone in type 1 diabetes: impact of immunosuppressive therapy on progression of diabetic nephropathy. *Diabetes Care* 2007; **30**: 1150-1155 [PMID: 17259471]

19 **Paroni R**, Fermo I, Fiorina P, Cighetti G. Determination of asymmetric and symmetric dimethylarginines in plasma

of hyperhomocysteinemic subjects. *Amino Acids* 2005; **28**: 389-394 [PMID: 15827687]
20 **Astorri E**, Fiorina P, Gavaruzzi G, Astorri A, Magnati G. Left ventricular function in insulin-dependent and in non-insulin-dependent diabetic patients: radionuclide assessment. *Cardiology* 1997; **88**: 152-155 [PMID: 9096915]
21 **Folli F**, Guzzi V, Perego L, Coletta DK, Finzi G, Placidi C, La Rosa S, Capella C, Socci C, Lauro D, Tripathy D, Jenkinson C, Paroni R, Orsenigo E, Cighetti G, Gregorini L, Staudacher C, Secchi A, Bachi A, Brownlee M, Fiorina P. Proteomics reveals novel oxidative and glycolytic mechanisms in type 1 diabetic patients' skin which are normalized by kidney-pancreas transplantation. *PLoS One* 2010; **5**: e9923 [PMID: 20360867]
22 **Sutherland DE**, Gruessner RW, Dunn DL, Matas AJ, Humar A, Kandaswamy R, Mauer SM, Kennedy WR, Goetz FC, Robertson RP, Gruessner AC, Najarian JS. Lessons learned from more than 1,000 pancreas transplants at a single institution. *Ann Surg* 2001; **233**: 463-501 [PMID: 11303130]
23 **Shapiro AM**, Ryan EA, Lakey JR. Clinical islet transplant--state of the art. *Transplant Proc* 2001; **33**: 3502-3503 [PMID: 11750497]
24 **Ryan EA**, Lakey JR, Paty BW, Imes S, Korbutt GS, Kneteman NM, Bigam D, Rajotte RV, Shapiro AM. Successful islet transplantation: continued insulin reserve provides long-term glycemic control. *Diabetes* 2002; **51**: 2148-2157 [PMID: 12086945]
25 **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]
26 **Vergani A**, D'Addio F, Jurewicz M, Petrelli A, Watanabe T, Liu K, Law K, Schuetz C, Carvello M, Orsenigo E, Deng S, Rodig SJ, Ansari JM, Staudacher C, Abdi R, Williams J, Markmann J, Atkinson M, Sayegh MH, Fiorina P. A novel clinically relevant strategy to abrogate autoimmunity and regulate alloimmunity in NOD mice. *Diabetes* 2010; **59**: 2253-2264 [PMID: 20805386 DOI: 10.2337/db09-1264]
27 **Williams PW**. Notes on diabetes treated with extract and by grafts of sheep's pancreas. *BMJ* 1894; **2**: 1303-1304
28 **Miszta-Lane H**, Mirbolooki M, James Shapiro AM, Lakey JR. Stem cell sources for clinical islet transplantation in type 1 diabetes: embryonic and adult stem cells. *Med Hypotheses* 2006; **67**: 909-913 [PMID: 16762516]
29 **Hirshberg B**, Rother KI, Digon BJ, Lee J, Gaglia JL, Hines K, Read EJ, Chang R, Wood BJ, Harlan DM. Benefits and risks of solitary islet transplantation for type 1 diabetes using steroid-sparing immunosuppression: the National Institutes of Health experience. *Diabetes Care* 2003; **26**: 3288-3295 [PMID: 14633816]
30 **Krishnan L**, Touroo J, Reed R, Boland E, Hoying JB, Williams SK. Vascularization and cellular isolation potential of a novel electrospun cell delivery vehicle. *J Biomed Mater Res A* 2014; **102**: 2208-2219 [PMID: 23913805 DOI: 10.1002/jbm.a.34900]
31 **Wagers AJ**, Weissman IL. Plasticity of adult stem cells. *Cell* 2004; **116**: 639-648 [PMID: 15006347]
32 **Zalzman M**, Gupta S, Giri RK, Berkovich I, Sappal BS, Karnieli O, Zern MA, Fleischer N, Efrat S. Reversal of hyperglycemia in mice by using human expandable insulin-producing cells differentiated from fetal liver progenitor cells. *Proc Natl Acad Sci USA* 2003; **100**: 7253-7258 [PMID: 12756298]
33 **Hori Y**, Gu X, Xie X, Kim SK. Differentiation of insulin-producing cells from human neural progenitor cells. *PLoS Med* 2005; **2**: e103 [PMID: 15839736]
34 **Kodama S**, Kühtreiber W, Fujimura S, Dale EA, Faustman DL. Islet regeneration during the reversal of autoimmune diabetes in NOD mice. *Science* 2003; **302**: 1223-1227 [PMID: 14615542]
35 **Ianus A**, Holz GG, Theise ND, Hussain MA. In vivo derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell fusion. *J Clin Invest* 2003; **111**: 843-850 [PMID: 12639990]
36 **Tang DQ**, Cao LZ, Burkhardt BR, Xia CQ, Litherland SA, Atkinson MA, Yang LJ. In vivo and in vitro characterization of insulin-producing cells obtained from murine bone marrow. *Diabetes* 2004; **53**: 1721-1732 [PMID: 15220196]
37 **Ramiya VK**, Maraist M, Arfors KE, Schatz DA, Peck AB, Cornelius JG. Reversal of insulin-dependent diabetes using islets generated in vitro from pancreatic stem cells. *Nat Med* 2000; **6**: 278-282 [PMID: 10700229]
38 **Bonner-Weir S**, Taneja M, Weir GC, Tatarkiewicz K, Song KH, Sharma A, O'Neil JJ. In vitro cultivation of human islets from expanded ductal tissue. *Proc Natl Acad Sci USA* 2000; **97**: 7999-8004 [PMID: 10884429]
39 **Fiorina P**, Voltarelli J, Zavazava N. Immunological applications of stem cells in type 1 diabetes. *Endocr Rev* 2011; **32**: 725-754 [PMID: 21862682 DOI: 10.1210/er.2011-0008]
40 **Francese R**, Fiorina P. Immunological and regenerative properties of cord blood stem cells. *Clin Immunol* 2010; **136**: 309-322 [PMID: 20447870 DOI: 10.1016/j.clim.2010.04.010]
41 **Parekh VS**, Joglekar MV, Hardikar AA. Differentiation of human umbilical cord blood-derived mononuclear cells to endocrine pancreatic lineage. *Differentiation* 2009; **78**: 232-240 [PMID: 19664871 DOI: 10.1016/j.diff.2009.07.004]
42 **Ende N**, Chen R, Reddi AS. Effect of human umbilical cord blood cells on glycemia and insulitis in type 1 diabetic mice. *Biochem Biophys Res Commun* 2004; **325**: 665-669 [PMID: 15541340]
43 **Fedyunina IA**, Rzhaninova AA, Kirienko EE, Goldshtein DV. Isolation of insulin-producing cells from different populations of multipotent stromal cells of the umbilical cord and human adipose tissue. *Bull Exp Biol Med* 2011; **151**: 114-120 [PMID: 22442815]
44 **Chandra V**, G S, Phadnis S, Nair PD, Bhonde RR. Generation of pancreatic hormone-expressing islet-like cell aggregates from murine adipose tissue-derived stem cells. *Stem Cells* 2009; **27**: 1941-1953 [PMID: 19544426 DOI: 10.1002/stem.117]
45 **Zuk PA**, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell* 2002; **13**: 4279-4295 [PMID: 12475952]
46 **Schäffler A**, Büchler C. Concise review: adipose tissue-derived stromal cells--basic and clinical implications for novel cell-based therapies. *Stem Cells* 2007; **25**: 818-827 [PMID: 17420225]
47 **Doi K**, Tanaka S, Iida H, Eto H, Kato H, Aoi N, Kuno S, Hirohi T, Yoshimura K. Stromal vascular fraction isolated from lipo-aspirates using an automated processing system: bench and bed analysis. *J Tissue Eng Regen Med* 2013; **7**: 864-870 [PMID: 22438241 DOI: 10.1002/term.1478]
48 **Gimble JM**, Guilak F, Bunnell BA. Clinical and preclinical translation of cell-based therapies using adipose tissue-derived cells. *Stem Cell Res Ther* 2010; **1**: 19 [PMID: 20587076 DOI: 10.1186/scrt19]
49 **De Ugarte DA**, Morizono K, Elbarbary A, Alfonso Z, Zuk PA, Zhu M, Dragoo JL, Ashjian P, Thomas B, Benhaim P, Chen I, Fraser J, Hedrick MH. Comparison of multi-lineage cells from human adipose tissue and bone marrow. *Cells Tissues Organs* 2003; **174**: 101-109 [PMID: 12835573]
50 **Lee RH**, Kim B, Choi I, Kim H, Choi HS, Suh K, Bae YC, Jung JS. Characterization and expression analysis of mesenchymal stem cells from human bone marrow and adipose tissue. *Cell Physiol Biochem* 2004; **14**: 311-324 [PMID: 15319535]
51 **Dicker A**, Le Blanc K, Aström G, van Harmelen V, Götherström C, Blomqvist L, Arner P, Rydén M. Functional studies of mesenchymal stem cells derived from adult human adipose tissue. *Exp Cell Res* 2005; **308**: 283-290 [PMID: 15925364]
52 **Fraser J**, Wulur I, Alfonso Z, Zhu M, Wheeler E. Differences in stem and progenitor cell yield in different subcutaneous adipose tissue depots. *Cytotherapy* 2007; **9**: 459-467 [PMID:

17786607]
53 **Strem BM**, Hicok KC, Zhu M, Wulur I, Alfonso Z, Schreiber RE, Fraser JK, Hedrick MH. Multipotential differentiation of adipose tissue-derived stem cells. *Keio J Med* 2005; **54**: 132-141 [PMID: 16237275]
54 **Nakao N**, Nakayama T, Yahata T, Muguruma Y, Saito S, Miyata Y, Yamamoto K, Naoe T. Adipose tissue-derived mesenchymal stem cells facilitate hematopoiesis in vitro and in vivo: advantages over bone marrow-derived mesenchymal stem cells. *Am J Pathol* 2010; **177**: 547-554 [PMID: 20558580 DOI: 10.2353/ajpath.2010.091042]
55 **Kodama S**, Faustman DL. Routes to regenerating islet cells: stem cells and other biological therapies for type 1 diabetes. *Pediatr Diabetes* 2004; **5** Suppl 2: 38-44 [PMID: 15601373]
56 **Timper K**, Seboek D, Eberhardt M, Linscheid P, Christ-Crain M, Keller U, Müller B, Zulewski H. Human adipose tissue-derived mesenchymal stem cells differentiate into insulin, somatostatin, and glucagon expressing cells. *Biochem Biophys Res Commun* 2006; **341**: 1135-1140 [PMID: 16460677]
57 **Ahlgren U**, Pfaff SL, Jessell TM, Edlund T, Edlund H. Independent requirement for ISL1 in formation of pancreatic mesenchyme and islet cells. *Nature* 1997; **385**: 257-260 [PMID: 9000074]
58 **Lechner A**, Leech CA, Abraham EJ, Nolan AL, Habener JF. Nestin-positive progenitor cells derived from adult human pancreatic islets of Langerhans contain side population (SP) cells defined by expression of the ABCG2 (BCRP1) ATP-binding cassette transporter. *Biochem Biophys Res Commun* 2002; **293**: 670-674 [PMID: 12054520]
59 **Cai J**, Cheng A, Luo Y, Lu C, Mattson MP, Rao MS, Furukawa K. Membrane properties of rat embryonic multipotent neural stem cells. *J Neurochem* 2004; **88**: 212-226 [PMID: 14675165]
60 **Kojima H**, Fujimiya M, Matsumura K, Nakahara T, Hara M, Chan L. Extrapancreatic insulin-producing cells in multiple organs in diabetes. *Proc Natl Acad Sci USA* 2004; **101**: 2458-2463 [PMID: 14983031]
61 **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]
62 **Hori Y**, Rulifson IC, Tsai BC, Heit JJ, Cahoy JD, Kim SK. Growth inhibitors promote differentiation of insulin-producing tissue from embryonic stem cells. *Proc Natl Acad Sci USA* 2002; **99**: 16105-16110 [PMID: 12441403]
63 **Soria B**, Roche E, Berná G, León-Quinto T, Reig JA, Martín F. Insulin-secreting cells derived from embryonic stem cells normalize glycemia in streptozotocin-induced diabetic mice. *Diabetes* 2000; **49**: 157-162 [PMID: 10868930]
64 **Paek HJ**, Morgan JR, Lysaght MJ. Sequestration and synthesis: the source of insulin in cell clusters differentiated from murine embryonic stem cells. *Stem Cells* 2005; **23**: 862-867 [PMID: 15888689]
65 **Paek HJ**, Moise LJ, Morgan JR, Lysaght MJ. Origin of insulin secreted from islet-like cell clusters derived from murine embryonic stem cells. *Cloning Stem Cells* 2005; **7**: 226-231 [PMID: 16390258]
66 **Dave SD**, Vanikar AV, Trivedi HL. Ex vivo generation of glucose sensitive insulin secreting mesenchymal stem cells derived from human adipose tissue. *Indian J Endocrinol Metab* 2012; **16** Suppl 1: S65-S69 [PMID: 22701849 DOI: 10.4103/2230-8210.94264]
67 **Chandra V**, Swetha G, Muthyala S, Jaiswal AK, Bellare JR, Nair PD, Bhonde RR. Islet-like cell aggregates generated from human adipose tissue derived stem cells ameliorate experimental diabetes in mice. *PLoS One* 2011; **6**: e20615 [PMID: 21687731 DOI: 10.1371/journal.pone.0020615]
68 **Zhang S**, Dai H, Wan N, Moore Y, Dai Z. Promoting long-term survival of insulin-producing cell grafts that differentiate from adipose tissue-derived stem cells to cure type 1 diabetes. *PLoS One* 2011; **6**: e29706 [PMID: 22216347 DOI: 10.1371/journal.pone.0029706]
69 **Lee J**, Kim SC, Kim SJ, Lee H, Jung EJ, Jung SH, Han DJ. Differentiation of human adipose tissue-derived stem cells into aggregates of insulin-producing cells through the over-expression of pancreatic and duodenal homeobox gene-1. *Cell Transplant* 2013; **22**: 1053-1060 [PMID: 23031216 DOI: 10.3727/096368912X657215]
70 **Dor Y**, Brown J, Martinez OI, Melton DA. Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. *Nature* 2004; **429**: 41-46 [PMID: 15129273]
71 **Baeyens L**, De Breuck S, Lardon J, Mfopou JK, Rooman I, Bouwens L. In vitro generation of insulin-producing beta cells from adult exocrine pancreatic cells. *Diabetologia* 2005; **48**: 49-57 [PMID: 15616797]
72 **Minami K**, Okuno M, Miyawaki K, Okumachi A, Ishizaki K, Oyama K, Kawaguchi M, Ishizuka N, Iwanaga T, Seino S. Lineage tracing and characterization of insulin-secreting cells generated from adult pancreatic acinar cells. *Proc Natl Acad Sci USA* 2005; **102**: 15116-15121 [PMID: 16210247]
73 **Seaberg RM**, Smukler SR, Kieffer TJ, Enikolopov G, Asghar Z, Wheeler MB, Korbutt G, van der Kooy D. Clonal identification of multipotent precursors from adult mouse pancreas that generate neural and pancreatic lineages. *Nat Biotechnol* 2004; **22**: 1115-1124 [PMID: 15322557]
74 **Zulewski H**, Abraham EJ, Gerlach MJ, Daniel PB, Moritz W, Müller B, Vallejo M, Thomas MK, Habener JF. Multipotential nestin-positive stem cells isolated from adult pancreatic islets differentiate ex vivo into pancreatic endocrine, exocrine, and hepatic phenotypes. *Diabetes* 2001; **50**: 521-533 [PMID: 11246871]
75 **Korsgren O**, Lundgren T, Felldin M, Foss A, Isaksson B, Permert J, Persson NH, Rafael E, Rydén M, Salmela K, Tibell A, Tufveson G, Nilsson B. Optimising islet engraftment is critical for successful clinical islet transplantation. *Diabetologia* 2008; **51**: 227-232 [PMID: 18040664]
76 **Lifson N**, Lassa CV, Dixit PK. Relation between blood flow and morphology in islet organ of rat pancreas. *Am J Physiol* 1985; **249**: E43-E48 [PMID: 2409813]
77 **Carlsson PO**, Liss P, Andersson A, Jansson L. Measurements of oxygen tension in native and transplanted rat pancreatic islets. *Diabetes* 1998; **47**: 1027-1032 [PMID: 9648824]
78 **Barshes NR**, Wyllie S, Goss JA. Inflammation-mediated dysfunction and apoptosis in pancreatic islet transplantation: implications for intrahepatic grafts. *J Leukoc Biol* 2005; **77**: 587-597 [PMID: 15728243]
79 **Ohmura Y**, Tanemura M, Kawaguchi N, Machida T, Tanida T, Deguchi T, Wada H, Kobayashi S, Marubashi S, Eguchi H, Takeda Y, Matsuura N, Ito T, Nagano H, Doki Y, Mori M. Combined transplantation of pancreatic islets and adipose tissue-derived stem cells enhances the survival and insulin function of islet grafts in diabetic mice. *Transplantation* 2010; **90**: 1366-1373 [PMID: 21076379 DOI: 10.1097/TP.0b013e3181ffba31]
80 **Moon MH**, Kim SY, Kim YJ, Kim SJ, Lee JB, Bae YC, Sung SM, Jung JS. Human adipose tissue-derived mesenchymal stem cells improve postnatal neovascularization in a mouse model of hindlimb ischemia. *Cell Physiol Biochem* 2006; **17**: 279-290 [PMID: 16791003]
81 **Kim Y**, Kim H, Cho H, Bae Y, Suh K, Jung J. Direct comparison of human mesenchymal stem cells derived from adipose tissues and bone marrow in mediating neovascularization in response to vascular ischemia. *Cell Physiol Biochem* 2007; **20**: 867-876 [PMID: 17982269]
82 **Rehman J**, Traktuev D, Li J, Merfeld-Clauss S, Temm-Grove CJ, Bovenkerk JE, Pell CL, Johnstone BH, Considine RV, March KL. Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. *Circulation* 2004; **109**: 1292-1298 [PMID: 14993122]
83 **Ghannam S**, Bouffi C, Djouad F, Jorgensen C, Noël D. Im-

munosuppression by mesenchymal stem cells: mechanisms and clinical applications. *Stem Cell Res Ther* 2010; **1**: 2 [PMID: 20504283 DOI: 10.1186/scrt2]
84 **Keyser KA**, Beagles KE, Kiem HP. Comparison of mesenchymal stem cells from different tissues to suppress T-cell activation. *Cell Transplant* 2007; **16**: 555-562 [PMID: 17708345]
85 **González MA**, Gonzalez-Rey E, Rico L, Büscher D, Delgado M. Treatment of experimental arthritis by inducing immune tolerance with human adipose-derived mesenchymal stem cells. *Arthritis Rheum* 2009; **60**: 1006-1019 [PMID: 19333946 DOI: 10.10002/art24405]
86 **Constantin G**, Marconi S, Rossi B, Angiari S, Calderan L, Anghileri E, Gini B, Bach SD, Martinello M, Bifari F, Galiè M, Turano E, Budui S, Sbarbati A, Krampera M, Bonetti B. Adipose-derived mesenchymal stem cells ameliorate chronic experimental autoimmune encephalomyelitis. *Stem Cells* 2009; **27**: 2624-2635 [PMID: 19676124 DOI: 10.1002/stem.194]
87 **Kang JW**, Kang KS, Koo HC, Park JR, Choi EW, Park YH. Soluble factors-mediated immunomodulatory effects of canine adipose tissue-derived mesenchymal stem cells. *Stem Cells Dev* 2008; **17**: 681-693 [PMID: 18717642 DOI: 10.1089/scd.2007.0153]
88 **Cui L**, Yin S, Liu W, Li N, Zhang W, Cao Y. Expanded adipose-derived stem cells suppress mixed lymphocyte reaction by secretion of prostaglandin E2. *Tissue Eng* 2007; **13**: 1185-1195 [PMID: 17518704]
89 **Vériter S**, Gianello P, Igarashi Y, Beaurin G, Ghyselinck A, Aouassar N, Jordan B, Gallez B, Dufrane D. Improvement of Subcutaneous Bioartificial Pancreas Vascularization and Function by Co-Encapsulation of Pig Islets and Mesenchymal Stem Cells in Primates. *Cell Transplant* 2013; Epub ahead of print [PMID: 23461890 DOI: 10.3727/096368913X663550]
90 **Evgenov NV**, Medarova Z, Pratt J, Pantazopoulos P, Leyting S, Bonner-Weir S, Moore A. In vivo imaging of immune rejection in transplanted pancreatic islets. *Diabetes* 2006; **55**: 2419-2428 [PMID: 16936189]
91 **Barshes NR**, Lee T, Goodpasture S, Brunicardi FC, Alejandro R, Ricordi C, Soltes G, Barth M, Hamilton D, Goss JA. Achievement of insulin independence via pancreatic islet transplantation using a remote isolation center: a first-year review. *Transplant Proc* 2004; **36**: 1127-1129 [PMID: 15194393]
92 **Cavallari G**, Olivi E, Bianchi F, Neri F, Foroni L, Valente S, La Manna G, Nardo B, Stefoni S, Ventura C. Mesenchymal stem cells and islet cotransplantation in diabetic rats: improved islet graft revascularization and function by human adipose tissue-derived stem cells preconditioned with natural molecules. *Cell Transplant* 2012; **21**: 2771-2781 [PMID: 22472472 DOI: 10.3727/096368912X637046]
93 **Terada N**, Hamazaki T, Oka M, Hoki M, Mastalerz DM, Nakano Y, Meyer EM, Morel L, Petersen BE, Scott EW. Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. *Nature* 2002; **416**: 542-545 [PMID: 11932747]
94 **Jiang Y**, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M, Du J, Aldrich S, Lisberg A, Low WC, Largaespada DA, Verfaillie CM. Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature* 2002; **418**: 41-49 [PMID: 12077603]
95 **Weissman IL**, Anderson DJ, Gage F. Stem and progenitor cells: origins, phenotypes, lineage commitments, and transdifferentiations. *Annu Rev Cell Dev Biol* 2001; **17**: 387-403 [PMID: 11687494]
96 **Ishihara H**, Maechler P, Gjinovci A, Herrera PL, Wollheim CB. Islet beta-cell secretion determines glucagon release from neighbouring alpha-cells. *Nat Cell Biol* 2003; **5**: 330-335 [PMID: 12640462]
97 **Kleinman R**, Ohning G, Wong H, Watt P, Walsh J, Brunicardi FC. Regulatory role of intraislet somatostatin on insulin secretion in the isolated perfused human pancreas. *Pancreas* 1994; **9**: 172-178 [PMID: 7910686]
98 **Creutzfeldt W**. The entero-insular axis in type 2 diabetes--incretins as therapeutic agents. *Exp Clin Endocrinol Diabetes* 2001; **109** Suppl 2: S288-S303 [PMID: 11460578]
99 **Holst JJ**, Vilsbøll T, Deacon CF. The incretin system and its role in type 2 diabetes mellitus. *Mol Cell Endocrinol* 2009; **297**: 127-136 [PMID: 18786605 DOI: 10.1016/j.mce.2008.08.012]
100 **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]
101 **Gault VA**, Kerr BD, Harriott P, Flatt PR. Administration of an acylated GLP-1 and GIP preparation provides added beneficial glucose-lowering and insulinotropic actions over single incretins in mice with Type 2 diabetes and obesity. *Clin Sci* (Lond) 2011; **121**: 107-117 [PMID: 21332446 DOI: 10.1042/CS20110006]
102 **Pørksen N**. The in vivo regulation of pulsatile insulin secretion. *Diabetologia* 2002; **45**: 3-20 [PMID: 11845219]
103 **Suschek C**, Fehsel K, Kröncke KD, Sommer A, Kolb-Bachofen V. Primary cultures of rat islet capillary endothelial cells. Constitutive and cytokine-inducible macrophagelike nitric oxide synthases are expressed and activities regulated by glucose concentration. *Am J Pathol* 1994; **145**: 685-695 [PMID: 7521579]
104 **Brissova M**, Fowler M, Wiebe P, Shostak A, Shiota M, Radhika A, Lin PC, Gannon M, Powers AC. Intraislet endothelial cells contribute to revascularization of transplanted pancreatic islets. *Diabetes* 2004; **53**: 1318-1325 [PMID: 15111502]
105 **Ahrén B**, Holst JJ. The cephalic insulin response to meal ingestion in humans is dependent on both cholinergic and noncholinergic mechanisms and is important for postprandial glycemia. *Diabetes* 2001; **50**: 1030-1038 [PMID: 11334405]
106 **Secchi A**, Caldara R, Caumo A, Monti LD, Bonfatti D, Di Carlo V, Pozza G. Cephalic-phase insulin and glucagon release in normal subjects and in patients receiving pancreas transplantation. *Metabolism* 1995; **44**: 1153-1158 [PMID: 7666788]
107 **Berthoud HR**, Trimble ER, Siegel EG, Bereiter DA, Jeanrenaud B. Cephalic-phase insulin secretion in normal and pancreatic islet-transplanted rats. *Am J Physiol* 1980; **238**: E336-E340 [PMID: 6769337]
108 **Konno M**, Hamabe A, Hasegawa S, Ogawa H, Fukusumi T, Nishikawa S, Ohta K, Kano Y, Ozaki M, Noguchi Y, Sakai D, Kudoh T, Kawamoto K, Eguchi H, Satoh T, Tanemura M, Nagano H, Doki Y, Mori M, Ishii H. Adipose-derived mesenchymal stem cells and regenerative medicine. *Dev Growth Differ* 2013; **55**: 309-318 [PMID: 23452121 DOI: 10.1111/dgd.12049]
109 **Kajiyama H**, Hamazaki TS, Tokuhara M, Masui S, Okabayashi K, Ohnuma K, Yabe S, Yasuda K, Ishiura S, Okochi H, Asashima M. Pdx1-transfected adipose tissue-derived stem cells differentiate into insulin-producing cells in vivo and reduce hyperglycemia in diabetic mice. *Int J Dev Biol* 2010; **54**: 699-705 [PMID: 19757377 DOI: 10.1387/ijdb.092953hk]
110 **Navarro X**, Sutherland DE, Kennedy WR. Long-term effects of pancreatic transplantation on diabetic neuropathy. *Ann Neurol* 1997; **42**: 727-736 [PMID: 9392572]
111 **Fioretto P**, Steffes MW, Sutherland DE, Goetz FC, Mauer M. Reversal of lesions of diabetic nephropathy after pancreas transplantation. *N Engl J Med* 1998; **339**: 69-75 [PMID: 9654536]
112 **Kim HK**, Kim YJ, Kim JT, Kwon CH, Kim YK, Bae YC, Kim DH, Jung JS. Alterations in the proangiogenic functions of adipose tissue-derived stromal cells isolated from diabetic rats. *Stem Cells Dev* 2008; **17**: 669-680 [PMID: 18788931 DOI: 10.1089/scd.2008.0141]
113 **Ryu S**, Kodama S, Ryu K, Schoenfeld DA, Faustman DL. Reversal of established autoimmune diabetes by restoration of endogenous beta cell function. *J Clin Invest* 2001; **108**: 63-72 [PMID: 11435458]
114 **Hess D**, Li L, Martin M, Sakano S, Hill D, Strutt B, Thyssen

S, Gray DA, Bhatia M. Bone marrow-derived stem cells initiate pancreatic regeneration. *Nat Biotechnol* 2003; **21**: 763-770 [PMID: 12819790]

115 **Vanikar AV**, Trivedi HL. Stem cell transplantation in living donor renal transplantation for minimization of immunosuppression. *Transplantation* 2012; **94**: 845-850 [PMID: 22992765 DOI: 10.1097/TP.0b013e3182664000]

116 **Kuo YR**, Chen CC, Goto S, Lee IT, Huang CW, Tsai CC, Wang CT, Chen CL. Modulation of immune response and T-cell regulation by donor adipose-derived stem cells in a rodent hind-limb allotransplant model. *Plast Reconstr Surg* 2011; **128**: 661e-672e [PMID: 22094768 DOI: 10.1097/PRS.0b013e318230c60b]

117 **Vanikar AV**, Trivedi HL, Feroze A, Kanodia KV, Dave SD, Shah PR. Effect of co-transplantation of mesenchymal stem cells and hematopoietic stem cells as compared to hematopoietic stem cell transplantation alone in renal transplantation to achieve donor hypo-responsiveness. *Int Urol Nephrol* 2011; **43**: 225-232 [PMID: 20084457 DOI: 10.1007/s11255-009-9659-1]

118 **Nam JS**, Kang HM, Kim J, Park S, Kim H, Ahn CW, Park JO, Kim KR. Transplantation of insulin-secreting cells differentiated from human adipose tissue-derived stem cells into type 2 diabetes mice. *Biochem Biophys Res Commun* 2014; **443**: 775-781 [PMID: 24148246 DOI: 10.1016/j.bbrc.2013.10.059]

119 **Zhao Y**, Jiang Z, Zhao T, Ye M, Hu C, Zhou H, Yin Z, Chen Y, Zhang Y, Wang S, Shen J, Thaker H, Jain S, Li Y, Diao Y, Chen Y, Sun X, Fisk MB, Li H. Targeting insulin resistance in type 2 diabetes via immune modulation of cord blood-derived multipotent stem cells (CB-SCs) in stem cell educator therapy: phase I/II clinical trial. *BMC Med* 2013; **11**: 160 [PMID: 23837842 DOI: 10.1186/1741-7015-11-160]

120 **Hao H**, Liu J, Shen J, Zhao Y, Liu H, Hou Q, Tong C, Ti D, Dong L, Cheng Y, Mu Y, Liu J, Fu X, Han W. Multiple intravenous infusions of bone marrow mesenchymal stem cells reverse hyperglycemia in experimental type 2 diabetes rats. *Biochem Biophys Res Commun* 2013; **436**: 418-423 [PMID: 23770360 DOI: 10.1016/j.bbrc.2013.05.117]

121 **Jiang R**, Han Z, Zhuo G, Qu X, Li X, Wang X, Shao Y, Yang S, Han ZC. Transplantation of placenta-derived mesenchymal stem cells in type 2 diabetes: a pilot study. *Front Med* 2011; **5**: 94-100 [PMID: 21681681 DOI: 10.1007/s11684-011-011-z]

122 **Estrada EJ**, Valacchi F, Nicora E, Brieva S, Esteve C, Echevarria L, Froud T, Bernetti K, Cayetano SM, Velazquez O, Alejandro R, Ricordi C. Combined treatment of intrapancreatic autologous bone marrow stem cells and hyperbaric oxygen in type 2 diabetes mellitus. *Cell Transplant* 2008; **17**: 1295-1304 [PMID: 19364067]

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TOPIC HIGHLIGHT

WJD 5th Anniversary Special Issues (1): Insulin

Defect of insulin signal in peripheral tissues: Important role of ceramide

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Abstract

In healthy people, balance between glucose production and its utilization is precisely controlled. When circulating glucose reaches a critical threshold level, pancreatic β cells secrete insulin that has two major actions: to lower circulating glucose levels by facilitating its uptake mainly into skeletal muscle while inhibiting its production by the liver. Interestingly, dietary triglycerides are the main source of fatty acids to fulfill energy needs of oxidative tissues. Normally, the unconsumed fraction of excess of fatty acids is stored in lipid droplets that are localized in adipocytes to provide energy during fasting periods. Thus, adipose tissue acts as a trap for fatty acid excess liberated from plasma triglycerides. When the buffering action of adipose tissue to store fatty acids is impaired, fatty acids that build up in other tissues are metabolized as sphingolipid derivatives such as ceramides. Several studies suggest that ceramides are among the most active lipid second messengers to inhibit the insulin signaling pathway and this review describes the major role played by ceramide accumulation in the development of insulin resistance of peripherals tissues through the targeting of specific proteins of the insulin signaling pathway.

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Key words: Diabetes; Insulin resistance; Lipids; Insulin signaling; Triglycerides; Palmitate; Sphingolipid; Akt; Ceramide synthase; Protein phosphatase 2A; Protein kinase C ζ/λ

Core tip: Muscle and liver represent major sites for insulin-mediated glucose metabolism. The ability of insulin to promote its peripheral action is reduced significantly by excess of saturated fat that stimulates intracellular production of second-messenger lipids such as ceramide. Studies suggest that ceramide could be important contributors to lipotoxicity, as the inhibition of early steps its biosynthesis pathway has large beneficial effects in rodent models of obesity and diabetes. In this review, we describe mechanisms by which ceramide acts on insulin-sensitive tissues and we propose that targeting this lipid family could be an interesting approach to fight diabetes.

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DIABETES EPIDEMIC

Diabetes has become a serious public health problem in

both developed and developing countries. Indeed, there is a dramatic increasing incidence of diabetes in most of these countries. In 2005, 217 million people worldwide had diabetes, and the World Health Organisation predicts that it will increase to 366 million in 2030[1]. In 2050, 33% of the population of the United States will suffer from diabetes[2]. One consequence is that over the years, diabetes has become life-threatening, with increased risk of cardiovascular diseases, retinopathy, kidney failure, and nerve and artery damages[3]. Diabetes is one of the first causes of haemodialysis, of blindness and of non-traumatic amputation of the legs. Another consequence is the increasing of health spending due to diabetes. For example, in the United States, diabetes costing is actually evaluated to more than $174 billion per year and it's expected to increase in subsequent years[2].

PATHOPHYSIOLOGY OF TYPE 2 DIABETES

There are different types of diabetes: (1) type 1 diabetes or maturity onset diabetes of the young associated to impairment of insulin production; and (2) type 2 diabetes, corresponding to 85%-90% of all diabetes, with both insulin secretion defects and peripheral insulin resistance. Type 2 diabetes is associated with obesity and although genetic factors play a role in the pathophysiology of this disease, other environmental factors such as diet and physical activity both play large roles. Several mechanisms have been proposed to explain both insulin resistance and insulin secretion defects observed in type 2 diabetes. Lipotoxicity, glucotoxicity, low grad systemic inflammation, oxidative stress and endoplasmic reticulum stress[4-6] correspond to different mechanisms that converge on a common pathway to induce insulin resistance. In this review we will focus on cellular lipid toxicity, *i.e.*, lipotoxicity.

LIPOTOXICITY

Systemic lipid imbalances are common in metabolic syndrome, in pre-diabetes and in type 2 diabetes and it is now clear that lipotoxicity can induce glucose dysregulation and participate to the pathophysiology of type 2 diabetes[7-9]. For example, prospective epidemiological studies performed in population with low or high risk to develop type 2 diabetes have shown that high free fatty acid (FFA) concentrations in plasma are associated with the risk of incident type 2 diabetes[10-12].

A major characteristic of type 2 diabetes is the loss of the ability of pancreatic β cells to increase insulin secretion to maintain normoglycemia in the face of insulin resistance[13]. Because of genetic predisposition, β cells could be unable to compensate the insulin resistance induced by FFA, but chronic exposition of β cells to high levels of FFA could equally explain defects in β cell function and decreased mass observed in type 2 diabetes. Indeed, *in vitro* studies have shown that FFA are associated with a decrease of insulin expression, synthesis and processing[14-16]. Another mechanism that can explain insulin secretion dysfunction in type 2 diabetes is that high FFA levels in islets induce β cell death[17]. In this review, we will not deal with this topic but we will rather focus our message on lipid-induced peripheral insulin resistance. To more information on lipotoxicity in pancreatic beta cells, confer to the excellent review of Boslem *et al*[18].

Since skeletal muscle constitutes 40% of human body mass and is quantitatively the most important tissue in regard to insulin-stimulated glucose disposal, it is considered the main cellular target in the development of insulin resistance. Thus, most of the studies investigating mechanisms of lipotoxicity induced insulin resistance were mostly performed in muscle tissue.

In 1963, Randle *et al*[19] have postulated that a competition between glucose and fatty acids for their oxidation and uptake is responsible for the onset of insulin resistance in muscle and adipose tissue. *In vivo* studies performed in both rodents and humans confirmed such insulin resistance obtained after lipid infusion but they also demonstrated that, in opposite to Randle's hypothesis, insulin resistance induced by lipids was not secondary to decreased glycolysis[20]. Indeed, lipids act directly on insulin signaling, resulting in an inhibition of the translocation of the insulin sensitive glucose transporter GLUT4 to the plasma membrane in response to the hormone, with subsequent reduced glucose uptake[21-25]. In human, data clearly show a strong correlation between lipid intramuscular content and insulin resistance[26-28] and a cross-sectional analysis performed in young, normal weight and non-diabetic adults reveals that a better correlation exists between muscle insulin sensitivity, assessed by the hyperinsulinaemic-euglycaemic clamp technique, and intramyocellular lipid content rather than with circulating lipid levels, body mass index, fasting blood glucose and age[29].

Liver is another important organ implicated in insulin resistance and, like in muscle indirect data also suggest an inverse relationship between lipid liver content and insulin sensibility. Indeed, ectopic lipid accumulation in the liver, termed nonalcoholic fatty liver disease (NAFLD), is associated with insulin resistance. Interestingly, in an animal model of lipodystrophy with steatosis, but without increased visceral fat, lipid liver content is associated with insulin resistance. Insulin resistance is reversed after reduction of steatosis with liver transplantation or recombinant leptin treatment[30]. Such association between steatosis and insulin resistance has also been observed in patients with severe lipodystrophy with equally a good response to recombinant leptin therapy[31]. Similarly, hepatic specific overexpression of lipoprotein lipase leads specifically to hepatic steatosis and hepatic insulin resistance[32,33]. During type 2 diabetes, reduction of steatosis by caloric restriction, or gastric bypass, is associated with increased insulin sensibility independently of visceral fat mass reduction[34,35].

Strong evidence exists between ectopic lipid accumulation and insulin resistance. However, in some cases, like in the "athlete's paradox", there is a lack of correlation

between ectopic lipid accumulation and peripheral insulin resistance. Indeed, athletes display high insulin sensitivity but also present increased levels of intramuscular fatty acids[36]. Thus, it seems that ectopic accumulation of fatty acids in non-adipose tissues can only be used as markers for the onset of insulin resistance but cannot be considered as a direct cause. Even if they do not seem to be directly involved, fatty acids contribute to insulin resistance as they lead to the synthesis of many lipid derivative intermediates such as diacylglycerol (DAG) and ceramide.

Over the years, studies have provided conclusive proof that ceramide plays a key role in the progression of insulin resistance in insulin sensitive tissues, targeting and inhibiting specific actors of the insulin signaling pathway.

INSULIN SIGNALING PATHWAY AND METABOLIC FUNCTIONS

Insulin is a polypeptide hormone whose major physiological role is to control glucose homeostasis by stimulating glucose uptake into insulin sensitive tissues (skeletal muscle and adipose tissue) and by inhibiting glucose output from the liver[37]. Insulin consists of two polypeptide chains, a α chain of 21 amino acid residues linked by two disulfide bonds to a β chain of 30 amino acid residues. Insulin is produced in the β cells of the Islets of Langerhans found in the pancreas. It is initially synthesized as an immature single polypeptide chain of 110 amino acids called pre-proinsulin. Pre-proinsulin contains an N-terminal domain of 24 amino acids that acts to direct the polypeptide to the endoplasmic reticulum during translation. This domain is later cleaved to yield proinsulin. Proinsulin is transported to the secretory vesicles of the pancreatic β cells, where a proteolytic enzyme removes the central 35 residues of the peptide (termed the C-peptide) that connect α and β chains to produce insulin. Insulin is then released into the blood stream by exocytosis. Secretion of the hormone is regulated by the glucose abundance in the plasma.

In skeletal muscle, insulin promotes the uptake of glucose and its conversion into glycogen. This tissue is an important target of the hormone, representing the major site of glucose disposal *in vivo*[37] and is reported to mediate 70%-80% of whole body insulin-stimulated glucose transport[38]. In the liver, insulin stimulates the synthesis of glycogen while inhibiting gluconeogenesis and glycogenolysis, halting hepatic glucose output. In adipocytes, insulin promotes the uptake of glucose and its conversion into a glycerophosphate of which can be esterified by 3 fatty acids, allowing to form triglycerides for long term storage, whereas simultaneously inhibiting the lipolytic pathway[39]. In addition to glucose metabolism, insulin also regulates many other cellular processes including amino acid transport, lipogenesis, protein synthesis and mitogenesis.

The first step in the activation of the insulin signaling pathway is the binding of insulin with its membrane receptor, the insulin receptor (IR). IR is a heterotetrameric complex of two subunits: α-subunit, and β-subunit that possess a transmembrane domain and an intracellular part. Binding of insulin to α subunits of IR induces a rapid conformational change in the receptor. This in turn stimulates the intrinsic tyrosine kinase activity of the β subunit resulting in trans-autophosphorylation of tyrosine residues in the intracellular region of the β subunits[40]. As a result of this autophosphorylation, the IR becomes catalytically active and promotes the tyrosine phosphorylation of a number of cellular proteins including the IR Substrate (IRS) proteins.

IRS proteins are major physiological targets of the activated insulin receptor kinase. Six different IRS isoforms have been identified so far[41]. In skeletal muscle and adipose tissue, IRS1 is the isoform that mediate insulin signaling. In the liver, however, IRS2 is the one that drives insulin metabolic functions. In the pancreas, IRS2 is an important regulator of cell growth and regeneration[41]. Studies have also shown that both IRS3 and IRS4 can be activated in response to insulin and insulin-like growth factor 1 (IGF1)[42] and that IRS3 can mediate insulin signaling in adipocytes[42]. Mice lacking either IRS3 or IRS4, however, display no major phenotype, suggesting that neither isoform plays a direct role in controlling glucose metabolism[43,44] but may rather act as negative regulators of the IGF1 signaling pathway by suppressing the function of other IRS isoforms[45].

One key molecule that is activated by the IRSs in response to insulin is phosphoinositide-3-kinase (PI3K). PI3K is a lipid kinase, which phosphorylates the D3 position of the inositol ring within inositol lipids resulting in the generation of 3-phosphoinositides (*e.g.*, PI-3P, PI-3,4P_2, and PI-3,4,5P_3). Eight mammalian isoforms of PI3K exist and they are grouped into three classes on the basis of their substrate specificity and structure: class Ⅰ, class Ⅱ, and class Ⅲ. Only class I can phosphorylate phosphatidylinositol, 4, 5-bisphosphate (PIP_2)[46]. Following PI3K activation, PIP_3 is generated from the substrate PIP_2. PIP_3 binds a protein displaying a PH domain and called the 3-phosphoinositide-dependent protein Kinase 1 (PDK1). Activated-PDK1 triggers downstream targets such as protein kinase B (PKB/Akt)[47].

PKB/Akt also called Akt is the third central node activated by insulin. It plays a crucial role in mediating signaling effects on metabolism, cell growth and cell cycle[48,49]. PKB/Akt has three isoforms: PKBα/Akt1, ubiquitously expressed, PKBβ/Akt2 mostly present in insulin responsive tissues (liver, adipose tissue and muscle), and PKBγ/Akt3 predominant in the brain. PKBβ/Akt2 is the isoform implicated in the regulation of glucose metabolism since neither PKBα Akt1 nor PKBγ/Akt3 ablation affects glucose metabolism[50].

PKB/Akt is activated through PI3K-produced PIP_3 which binds its PH domain. Then, PKB/Akt is recruited to the plasma membrane where it is activated by phosphorylation on two critical sites: threonine 308 (T308) in the activation loop and serine 473 (S473) in the hydrophobic motif[51]. PDK1 phosphorylates PKB/Akt on T308.

The kinase that phosphorylates the S473 site is the complex mammalian target of rapamycin complex 2, a regulator of cell growth and proliferation[52].

PKB/Akt is highly activated within minutes following cell exposure to insulin to mediate the metabolic effects of the hormone[49,53].

Indeed, principle roles of PKB/Akt in insulin sensitive tissues are to: (1) Stimulate glucose uptake in muscle and adipose tissue; (2) Trigger glucose storage as glycogen in muscle and in the liver; (3) Stimulate the conversion of glucose excess into lipids in the liver; (4) Induce protein synthesis in muscle; (5) Inhibit glycogen breakdown in both muscle and liver; (6) Suppress liberation of free fatty acids from adipose tissue; (7) Inhibit *de novo* production of glucose in the liver; and (8) Impede protein breakdown in muscle (Figure 1).

Considering the crucial role PKB/Akt plays in mediating insulin metabolic actions in cells, impairing PKB/Akt activity represents the best way to compromise the whole system.

LIPID SECOND MESSENGER AND LOSS OF INSULIN SENSITIVITY

In pathological situations such as obesity and type 2 diabetes that are characterized by insulin resistance, ectopic fatty acid accumulation is increased due to reduced mitochondrial fatty acid oxidation and to enhanced fatty acid uptake[54-57]. This increased fat content inversely correlates with insulin sensitivity in skeletal muscle, liver and adipocytes[58-61].

Interestingly and depending on the degree of saturation, free fatty acid may exert different effects on insulin signaling. Studies have demonstrated that saturated fatty acids such as palmitate (16:0) and stearate (18:0) impair insulin sensitivity in muscle[62,63], whereas mono-unsaturated fatty acids or poly-unsaturated fatty acids have no effect or even enhance insulin action[64-66]. Although the exact reasons behind these differences are unclear, studies have suggested that unsaturated fatty acids may be preferentially targeted for triglyceride synthesis and storage, whilst saturated fatty acids may be used for synthesis of critical lipid intermediates such as DAG and ceramide. These two lipid second messengers have been demonstrated to mediate deleterious actions of saturated fatty acids on insulin signaling.

DAG AND INSULIN RESISTANCE

DAG is a glyceride consisting of two fatty acid chains covalently bonded to a glycerol molecule. DAG, intermediate of both triglyceride and phospholipid metabolism, is an important second messenger involved in intracellular signaling[67].

DAG has been shown to accumulate in insulin resistant liver[68,69] and studies have shown that intra-hepatic DAG is an important mediator of hepatic insulin resistance in obese people with nonalcoholic fatty liver disease[70,71]. Elevated DAG content and activation of protein kinase C (PKC)ε has been associated with hepatic insulin resistance and the involvement of this "lipid-activated pathway" has been validated through the use of antisense oligonucleotide against PKCε in rats. Knocking down PKCε expression in liver protected rats from lipid-induced hepatic insulin resistance, despite increase in hepatic lipid content[72].

Several studies have decrypted the mechanism by which DAG-activated PKCs inhibit insulin signaling in liver. They show that IRS proteins are likely to be PKC's preferential targets. DAG-activated PKCs inhibit IRSs activity through their phosphorylation on several serine residues, preventing consequently insulin activation of IRSs through their phosphorylation on tyrosine residues[73-75].

In muscle, however, data are contradictory. Itani *et al*[76] were the first to point out the positive association between DAG content and muscle insulin resistance by comparing a group of subject receiving a lipid infusion to a control group. Lipid infusion resulted in a 3-fold increase in total DAG content in muscle, and reduced insulin sensitivity. Straczkowski *et al*[77] observed that total muscle DAG concentrations were higher in obese compared to lean controls and lean offspring type 2 diabetics, and this increased DAG content was inversely related to insulin sensitivity. Other studies have also confirmed this correlation[78,79].

However, the association between DAG and muscle insulin resistance is still questioned. Indeed, Vistisen *et al*[80] performed muscle biopsies during glucose clamps and they observed a reduction in insulin sensitivity after lipid infusion, without any changes in muscle DAG content. These results were confirmed by Anastasiou *et al*[81] that compared obese type 2 diabetic patients to non-diabetics subjects and found no difference in muscle DAG content between the groups. Similarly, Perreault *et al*[82] compared insulin resistant obese patients to glucose tolerant obese patients and again found no difference in DAG content between the groups. Even more intriguing, Amati *el al*[83] observed a two-fold increase in DAG content in insulin sensitive human muscle biopsies compared to insulin resistant human muscle biopsies. More recently, the same group showed no difference in muscle DAG content between lean subjects compared to obese insulin resistance patients[84].

Altogether, and in opposite to liver, it seems that DAG does not appear to be a crucial player in the onset of insulin resistance in muscle, and maybe more investigations are needed to really be able to conclude.

CERAMIDE AND INSULIN RESISTANCE

Ceramide biosynthesis

One of the main sphingolipid that has been demonstrated to play a crucial role in insulin resistance is ceramide. During obesity, ceramide is mainly generated from long chain fatty acyl-CoAs[85,86], and has been shown to be toxic lipid when it accumulates in tissues during obesity[87-89].

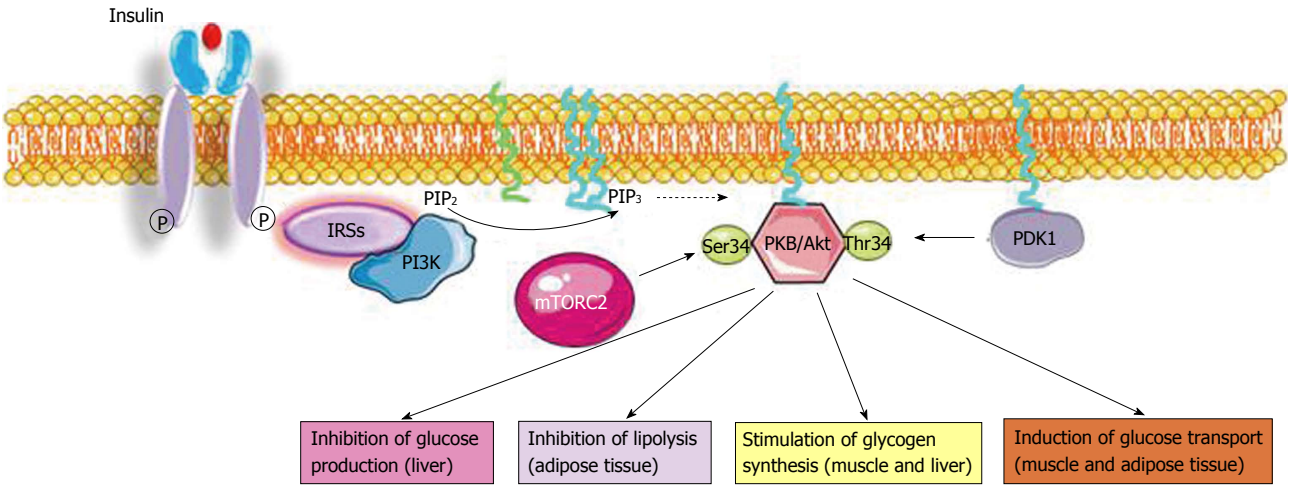

Figure 1 Insulin signaling pathway. Insulin binds with insulin receptor (IR) that activates the IR substrates (IRSs), the phosphoinositide-3-kinase (PI3K), and protein kinase B/Akt (PKBAkt). Activated PKB/Akt mediates insulin metabolic effects and regulates nutrients homeostasis. PIP_2: Phosphorylate phosphatidylinositol, 4, 5-bisphosphate; PDK1: 3-phosphoinositide-dependent protein kinase 1; mTORC2: Mammalian target of rapamycin complex 2.

Ceramide is a bioactive sphingolipid that has been implicated in mediating or regulating many cellular processes, including cell cycle arrest, proliferation, apoptosis, senescence, and stress responses. Ceramide plays also an important role in cell membrane structure[90].

Formation of ceramide can be induced by different stimuli such as tumor necrosis factor-α, heat stress, oxidative stress, ionizing radiation, and chemotherapeutics[91].

Multiple metabolic pathways converge to ceramide (Figure 2): (1) The *de novo* synthesis pathway from saturated fatty acids that takes place in the endoplasmic reticulum; (2) The sphingomyelinase pathway that uses sphingomyelinase to break down sphingomyelin in the cell membrane to release ceramide; and (3) The salvage pathway in lysosomes that occurs through breakdown of complex sphingolipids to give sphingosine, which is then rescued by reacylation to form ceramide.

In time of fatty acid plethora, the *de novo* ceramide biosynthesis pathway is the pathway that is likely to be most harnessed to synthesize ceramide. It occurs in the leaflet membrane of the endoplasmic reticulum where ceramide is synthesized through a series of reactions[92,93]. *De novo* synthesis of ceramide begins with the condensation of palmitate and serine to form 3-keto-dihydrosphingosine (Figure 2). This reaction is catalyzed by serine palmitoyl transferase (SPT) and is the rate-limiting step of the pathway. In turn, 3-keto-dihydrosphingosine is reduced to dihydrosphingosine, which is then followed by acylation by ceramide synthases (CerS) to produce dihydroceramide. In mammals, six CerS isoforms are expressed and are called CerS 1 to 6. They carry out the same chemical reaction, but display distinct specificities for the acyl-CoA chain length they use for N-acylation[94]. Thus, CerS isoforms are responsible for the fatty acid composition of ceramide. Interestingly, several studies have shown distinct cellular functions for ceramides with different N-acyl chain length[95,96]. The final reaction to produce ceramide is catalysed by dihydroceramide desaturase.

Inverse relationship between ceramide content and insulin sensitivity

Studies in animal and models: One of the early studies that analyzed ceramide content in obese Zucker fa/fa rats (rats homozygous for truncated, non-functional leptin receptor) was Turinsky *et al*[97] in 1990. The authors found that these rats present an increase in ceramide content in both muscle and liver. Increased ceramide content was also detected in insulin resistant models of rodents, as in ob/ob mice, mice fed on high fat diet, and in intra-lipid infused mice[85,98,99]. Altogether these reports illustrate the inverse relationship between ceramide and insulin sensitivity in rodent muscle. This association was also confirmed *in vitro* in cultured C2C12 and L6 myotubes, as well as in adipocytes[99-101]. Exposing cultured muscle cells to saturated fatty acids (like palmitate) attenuates insulin activation of glycogen synthesis and glucose transport concomitantly with increasing intracellular ceramide amounts[63,99]. Additionally, incubation of muscle cells and adipocytes with analogues of ceramide mimics the inhibitory effects of FFAs on insulin signaling and suppresses insulin-stimulated glycogen synthesis and glucose transport[100,101].

Studies in human subjects: In accordance with data obtained in rodents, studies in human subjects also support the inverse relationship between ceramide accumulation and insulin sensitivity. It has been shown that under basal conditions, total amount of ceramide in skeletal muscle is increased in obese subjects compared to lean ones[83,84,87]. Another study performed in human skeletal muscle of lean normoglycemic subjects revealed again an inverse relationship between muscle ceramide accumulation and insulin sensitivity[102]. The same authors show in another study a ceramide accumulation in muscle of type 2 diabetic patient offsprings compared to muscle of control subjects[77]. Furthermore, the group of Goodpaster demonstrated that physical exercise reduces ceramide

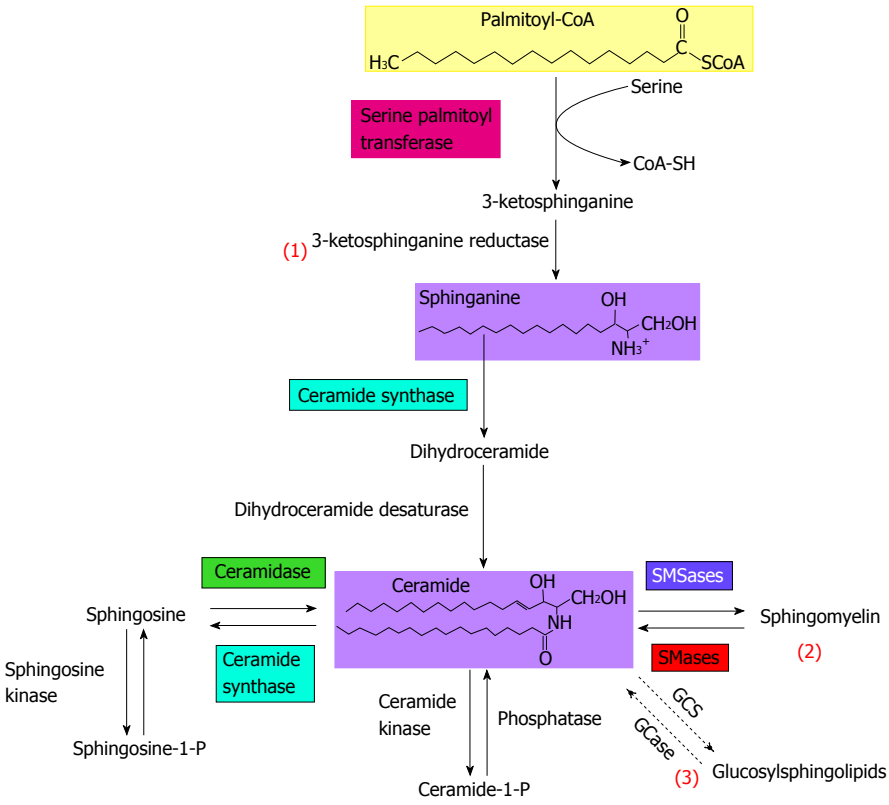

Figure 2 Sphingolipid metabolism. Ceramide can either be newly synthesized in *de novo* ceramide synthesis pathway (1), or it can be the product of complex sphingolipids degradation, including sphingomyelin hydrolysis (2). The degradation of glycosylsphingolipids constitutes the salvage pathway (3). GCase: Glucosyl ceramidase; GCS: Glucosylceramide synthase; SMases: Sphingomyelinases; SMSases: Sphingomyelin synthases.

content in obese and insulin resistant subjects, and this was correlated with improved insulin sensitivity[83,103]. Like in muscle, accumulation of ceramide content in human adipocytes has also been demonstrated to be related to insulin resistance[104,105].

Altogether, these studies prove a solid association between insulin resistance and an increase in ceramide content in both muscle and adipocytes.

Unlike in muscle and adipose cells, a role of ceramide in the onset of hepatic insulin resistance is more debated. Indeed, some studies see no ceramide accumulation in fatty liver[68,70,71], making improbable these lipids as mediators hepatic insulin resistance. This is in contradiction with another study showing increases in hamster hepatic ceramide levels in response to lipopolysaccharide administration[106]. In addition, Longato *et al*[107] saw a dysregulated ceramide metabolism in high fat diet-induced hepatic steatosis.

Interestingly, and in opposite to muscle and adipose tissue, ceramide cannot accumulate in the liver. Indeed, very recently, Watt *et al*[108] have shown that lipid infusion in healthy subjects resulted in a rapid hepatic secretion of ceramide in the circulation, primarily within very low-density lipoprotein[109,110], thereby protecting the liver from the deleterious effects of their intracellular accumulation. It would be interesting, however, to assess whether lipid-induced ceramide secretion is affected in fatty liver (steatosis).

Altogether, if ceramide does not seem to accumulate in liver during lipotoxic conditions, its secretion into the circulation could be deleterious for other peripheral tissues such as pancreatic β cells and muscle cells.

Implication of ceramide in the progression of insulin resistance

Two methods were used to validate the implication of ceramide in impaired insulin sensibility: the first one was to inhibit ceramide production, and the second was to enhance ceramide metabolism towards less harmful sphingolipid species.

Inhibition of ceramide production improves insulin sensitivity: One method used to demonstrate the role of ceramide in the onset of insulin resistance was to inhibit ceramide biosynthesis. The most commonly studied molecular target involved in suppressing ceramide production is the enzyme SPT, enzyme that catalyzes the initial rate-limiting step in *de novo* ceramide synthesis (Figure 3)[90]. Several potent inhibitors of SPT have been documented, although the most widely used is myriocin, a naturally occurring fungal metabolite isolated from Myriococcum albomyces[111]. In studies carried out *in vivo*, administration of myriocin was found to attenuate PKB/Akt inhibition in response to lipid infusion or high-fat feeding, as well as improving glucose tolerance and peripheral insulin sensitivity in obese ob/ob mice and Zucker Diabetic Fatty rats[112-114]. As expected, these beneficial effects of myriocin were associated with reduced

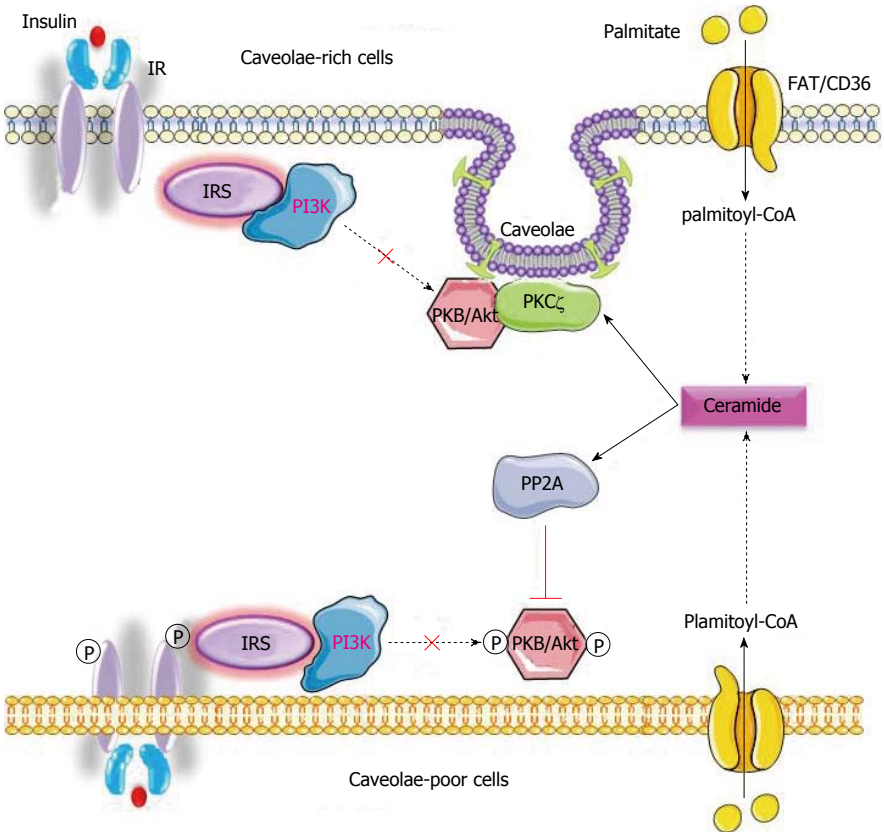

Figure 3 Ceramide inhibits insulin-induced activation of protein kinase B *via* two distinct mechanisms. Ceramide inhibits insulin-activation of protein kinase B (PKB/Akt) either by activating atypical PKC (PKCζ) or by stimulating the phosphatase PP2A. Activation of either mechanism depends on plasma membrane enrichment with caveolae: (1) Ceramide-activated PKCζ phosphorylates the PH domain of PKB/Akt on Thr/Ser34, changing the recognition site of PKB/Akt, and disabling its activation by PIP3; (2) PP2A dephosphorylates PKB/Akt, inhibiting its kinase activity. IR: Insulin receptor; IRS: IR substrates; PI3K: Phosphoinositide-3-kinase; PP2A: Protein phosphatase 2A.

levels of ceramide and were reproduced when alternative inhibitors of *de novo* ceramide synthesis such as L-cycloserine (which also inhibits SPT) and Fenretinide (dihydroceramide synthase inhibitor) were used[63,115].

Studies performed *in vitro* in myotubes confirmed what was observed *in vivo*. They demonstrated that acute inhibition of SPT using myriocin ameliorates the loss in insulin-stimulated PKB/Akt activation in cultured L6 or C2C12 myotubes caused by palmitate-driven ceramide synthesis[62,63].

Interestingly, a very recent study shows that inhibition of the *de novo* synthesis of ceramide using myriocin reduces hepatic lipid accumulation in liver of rats with NAFLD[116]. This inhibition of ceramide biosynthesis is accompanied with decreased in both DAG and triglyceride contents, resulting in amelioration of hepatic insulin resistance and improvement of glucose homeostasis[116].

Stimulation of ceramide conversion into less harmful sphingolipids improves insulin sensibility: The degradation of ceramide is initiated by the action of ceramidase that produces sphingosine, which is then phosphorylated to sphingosine-1-phosphate (S1P) by sphingosine kinase[117]. S1P is the final metabolic product of sphingolipid degradation and can function as an intracellular second messenger or in an autocrine and/or paracrine manner to activate and signal through S1P receptors[118]. Interestingly, S1P itself opposes the effects of ceramide on intracellular signaling. S1P has been shown to ameliorate insulin-stimulated glucose uptake, possibly through the activation of PKB/Akt[118-121]. Therefore, studies have aimed at finding ways to enhance ceramide metabolism into S1P in muscle in order to restore their insulin sensitivity. Bruce *et al*[122] used transgenic mice overexpressing sphingosine kinase. They show that high fat fed transgenic mice display improved insulin sensitivity compared to control mice. In addition, they used a drug called FTY720 which inhibits ceramide synthase activity and decrease ceramide accumulation in skeletal muscle[123]. As expected, they saw an improvement of insulin sensitivity. FTY720 prevented muscle ceramide accumulation in high fat fed mice and subsequently improved glucose homeostasis[124]. Other studies show that overexpression of ceramidase (converting ceramide to sphingosine) protects from lipid-induced muscle insulin resistance in C2C12 myotubes[125].

Altogether, these results demonstrate that preventing the aberrant accumulation of ceramide by promoting its metabolism into sphingosine and sphingosine-derivatives might restore normal insulin sensitivity and glucose me-

tabolism in models of insulin resistance.

Ceramide inhibitory effect on the insulin signaling pathway

Several studies have reported that ceramide may attenuate insulin-stimulated glucose transport and glycogen synthesis by antagonizing early events in insulin signaling such as activation of IRS-1[126] and possibly PI3K[127]. However, these results are controversial, as several groups reported no defects in the activation of these molecules upon challenging cells with ceramide[100,101]. In contrast, a number of groups suggested that PKB/Akt is the target of ceramide, and that inhibition of this kinase may account for reduced glucose transport and apoptosis observed in ceramide treated cells[99-101,128]. Consistent with this, defects in PKB/Akt activation have been noted in a variety of ceramide-treated cell types, including 3T3-L1 adipocytes[101], foetal brown adipocytes[129], L6 rat and C2C12 mouse skeletal muscle[99,100], A75R5 smooth muscle cells[130], and MCF7 breast cancer cells[131].

Furthermore, the inhibition of PKB/Akt by ceramide is not limited to experiments using exogenously supplied lipids. The hormonal activation of PKB/Akt is also blunted in muscle cells treated with free fatty acids in a manner which is dependent on the intracellular conversion of palmitate to ceramide[62,63,99]. Taken together these results suggest that ability of ceramide to impair PKB/Akt activity may be an important determinant of insulin sensitivity.

A key issue is the mechanism by which ceramide inhibits PKB/Akt activity. Depending on the cell enrichment in caveolin-enriched domain[132], ceramide inhibits the insulin-stimulated PKB/Akt either through the protein phosphatase 2A (PP2A), or *via* the atypical PKC (aPKC) pathway (Figure 3).

PP2A depended inhibition of insulin-induced activation of PKB/Akt: PP2A is a cytoplasmic serine/threonine phosphatase ubiquitously expressed that plays an important role in the regulation of diverse cellular processes, including metabolic enzymes, hormone receptors, kinase cascades, and cell growth[133]. It has been shown that insulin inhibits PP2A in physiologic conditions[134]. In contrast, several groups demonstrated that ceramide activates PP2A to promote the de-phosphorylation of PKB/Akt[62,135,136]. Two different inhibitors of PP2A activity, okadaic acid or SV40 small T antigen that binds with PP2A[137] were used to demonstrate the role of ceramide-induced PP2A inactivation of PKB/Akt. The presence of either inhibitor in cells treated with palmitate or short chain ceramide analogue (C2-ceramide), alleviated inhibition on PKB/Akt and re-established a normal, insulin signaling[62,128]. Therefore, one way for ceramide to inhibit PKB/Akt activity is by promoting its dephosphorylation at Thr308 and Ser473 through activation of PP2A.

Atypical PKCs another ceramide-stimulated protein altering PKB/Akt activation: The second mechanism of inactivation of PKB/Akt by ceramide requires the activation of aPKCs (PKCζ/λ). There is mounting evidence in the literature suggesting that aPKC may regulate PKB/Akt signaling and that the relationship between the two kinases may be subject to modulation by ceramide. It is 20 years since investigators first demonstrated that PKCζ/λ could associate with PKB/Akt in COS-7 fibroblasts[138]. It has also been demonstrated that PKCζ interacts directly with PKB/Akt in other cells types such as Chinese hamster ovary cells and COS-1 cells[139], as well as the BT-549 human breast cancer cell line[140].

In pathological conditions, ceramide-activated aPKCs impair insulin signaling. aPKCs phosphorylate PKB/Akt on its Thr34/Ser34 residue (Thr34 in PKBα and PKBβ, Ser34 in PKBγ), thus preventing PIP_3 to bind the kinase on its PH domain, and to translocate to the plasma membrane and its subsequent activation in response to insulin[132,141,142]. Based on these observations, it was proposed that an increase in intracellular ceramide leading to the activation of aPKCs promotes the stabilization of the aPKC-PKB/Akt complex and attenuates the recruitment of PKB/Akt to the plasma membrane as a result of disrupted PIP_3 binding (Figure 3).

CERAMIDE, A THERAPEUTIC TARGET?

Mechanisms by which saturated fatty acids act on insulin signaling are now getting clearer. They involve several lipid and protein intermediates that play an essential role to mediate the deleterious effects of accumulated saturated lipids in insulin sensitive tissues. Thus, two main options exist to counteract the action of these fatty acids on insulin signaling: (1) acting on ceramide downstream signaling targets (aPKCs or PP2A); or (2) modulating directly ceramide content[143]. Considering the large involvement of both aPKCs and PP2A in numerous paths[144,145], it would be more logical to try to directly inhibit the accumulation of ceramides in tissues. Several problems would arise with a complete inhibition of ceramide biosynthesis since these bioactive sphingolipids are in the center of sphingolipid metabolism. Indeed, ceramide signaling has been directly or indirectly involved in the diverse functions such as regulation of cell growth, differentiation, senescence, necrosis, proliferation, and apoptosis[90]. Therefore, inhibiting completely ceramide biosynthesis would be likely to be very harmful to the cells. Targeting specific ceramides species would be more appropriate since it has been shown that specific ceramide species could be associated with different functions, depending upon the cell type[94].

Concretely, it will be important to determine which ceramide species accumulate under lipotoxic conditions and then to evaluate whether these identified ceramide species enhance or reduce the deleterious effects of lipotoxicity in insulin sensitive tissues.

Interestingly, data existing already suggest that ceramide with distinct acyl chain-length are associated with different cell dysfunction in lipotoxic conditions. The

enzyme responsible of generating different ceramide acyl chain-length is the CerS. Six mammalian CerS have been described, with each utilizing fatty acyl CoAs of relatively defined chain lengths for ceramide synthesis[94]. In pancreatic β-cells, C18:0, C22:0 and C24:1 ceramides induce apoptosis, and inhibition of the CerS (CerS4) responsible for their synthesis blocks this phenomenon[146]. In the liver, CerS1 and CerS6, producing mainly C16:0 and C18:0 ceramides are associated with insulin resistance[147], whereas C22:0 and C24:0 ceramides produced through CerS2 are rather protective[148].

In muscle cells, however, no definitive and conclusive investigation has been carried out to date. The expression of C16:0, C18:0 and C24:0 ceramide species are increased in myotubes of type 2 diabetic patients compared to lean donors[149]. However, one recent paper shows that overexpression of each CerS isoform in L6 muscle cells does not point out any ceramide species in the generation of insulin resistance[150]. Since the implication of ceramide in the onset of insulin resistance in muscle has been convincingly demonstrated both *in vivo* and *in vitro* (see previous chapters), more investigations are needed before to make any conclusion in this tissue.

In summary, deciphering the mechanisms by which ceramides act negatively on insulin signaling has already been a step forward. However, the identification of the putative ceramide species that mediates lipotoxicity in cells or pushing ceramides to be converted into less toxic lipids remains the priority in order to find a way to counteract ceramide negative actions.

REFERENCES

1 **Smyth S**, Heron A. Diabetes and obesity: the twin epidemics. *Nat Med* 2006; **12**: 75-80 [PMID: 16397575 DOI: 10.1038/nm0106-75]

2 **Boyle JP**, Thompson TJ, Gregg EW, Barker LE, Williamson DF. Projection of the year 2050 burden of diabetes in the US adult population: dynamic modeling of incidence, mortality, and prediabetes prevalence. *Popul Health Metr* 2010; **8**: 29 [PMID: 20969750 DOI: 10.1186/1478-7954-8-29]

3 **White JR**. Economic considerations in treating patients with type 2 diabetes mellitus. *Am J Health Syst Pharm* 2002; **59** Suppl 9: S14-S17 [PMID: 12489382]

4 **Flamment M**, Hajduch E, Ferré P, Foufelle F. New insights into ER stress-induced insulin resistance. *Trends Endocrinol Metab* 2012; **23**: 381-390 [PMID: 22770719 DOI: 10.1016/j.tem.2012.06.003]

5 **Samuel VT**, Shulman GI. Mechanisms for insulin resistance: common threads and missing links. *Cell* 2012; **148**: 852-871 [PMID: 22385956 DOI: 10.1016/j.cell.2012.02.017]

6 **Mlinar B**, Marc J. New insights into adipose tissue dysfunction in insulin resistance. *Clin Chem Lab Med* 2011; **49**: 1925-1935 [PMID: 21892913 DOI: 10.1515/CCLM.2011.697]

7 **Reaven GM**. The fourth musketeer--from Alexandre Dumas to Claude Bernard. *Diabetologia* 1995; **38**: 3-13 [PMID: 7744226 DOI: 10.1007/BF02369347]

8 **Golay A**, Felber JP, Jequier E, DeFronzo RA, Ferrannini E. Metabolic basis of obesity and noninsulin-dependent diabetes mellitus. *Diabetes Metab Rev* 1988; **4**: 727-747 [PMID: 3069401]

9 **Abbasi F**, Carantoni M, Chen YD, Reaven GM. Further evidence for a central role of adipose tissue in the antihyperglycemic effect of metformin. *Diabetes Care* 1998; **21**: 1301-1305 [PMID: 9702437 DOI: 10.2337/diacare.21.8.1301]

10 **Charles MA**, Eschwège E, Thibult N, Claude JR, Warnet JM, Rosselin GE, Girard J, Balkau B. The role of non-esterified fatty acids in the deterioration of glucose tolerance in Caucasian subjects: results of the Paris Prospective Study. *Diabetologia* 1997; **40**: 1101-1106 [PMID: 9300248 DOI: 10.1007/s001250050793]

11 **Pankow JS**, Jacobs DR, Steinberger J, Moran A, Sinaiko AR. Insulin resistance and cardiovascular disease risk factors in children of parents with the insulin resistance (metabolic) syndrome. *Diabetes Care* 2004; **27**: 775-780 [PMID: 14988301 DOI: 10.2337/diacare.27.3.775]

12 **Paolisso G**, Gambardella A, Amato L, Tortoriello R, D'Amore A, Varricchio M, D'Onofrio F. Opposite effects of short- and long-term fatty acid infusion on insulin secretion in healthy subjects. *Diabetologia* 1995; **38**: 1295-1299 [PMID: 8582538]

13 **Klöppel G**, Löhr M, Habich K, Oberholzer M, Heitz PU. Islet pathology and the pathogenesis of type 1 and type 2 diabetes mellitus revisited. *Surv Synth Pathol Res* 1985; **4**: 110-125 [PMID: 3901180]

14 **Zhou YP**, Grill V. Long term exposure to fatty acids and ketones inhibits B-cell functions in human pancreatic islets of Langerhans. *J Clin Endocrinol Metab* 1995; **80**: 1584-1590 [PMID: 7745004 DOI: 10.1210/jc.80.5.1584]

15 **Lewis GF**. Lipid metabolism. *Curr Opin Lipidol* 2002; **13**: 97-99 [PMID: 11790968 DOI: 10.1097/00041433-200202000-00014]

16 **Chen NG**, Romsos DR. Enhanced sensitivity of pancreatic islets from preobese 2-week-old ob/ob mice to neurohormonal stimulation of insulin secretion. *Endocrinology* 1995; **136**: 505-511 [PMID: 7835283 DOI: 10.1210/en.136.2.505]

17 **Lupi R**, Dotta F, Marselli L, Del Guerra S, Masini M, Santangelo C, Patané G, Boggi U, Piro S, Anello M, Bergamini E, Mosca F, Di Mario U, Del Prato S, Marchetti P. Prolonged exposure to free fatty acids has cytostatic and pro-apoptotic effects on human pancreatic islets: evidence that beta-cell death is caspase mediated, partially dependent on ceramide pathway, and Bcl-2 regulated. *Diabetes* 2002; **51**: 1437-1442 [PMID: 11978640 DOI: 10.2337/diabetes.51.5.1437]

18 **Boslem E**, Meikle PJ, Biden TJ. Roles of ceramide and sphingolipids in pancreatic β-cell function and dysfunction. *Islets* 2012; **4**: 177-187 [PMID: 22847494 DOI: 10.4161/isl.20102]

19 **Randle PJ**, Garland PB, Hales CN, Newsholme EA. The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet* 1963; **1**: 785-789 [PMID: 13990765]

20 **Hue L**, Taegtmeyer H. The Randle cycle revisited: a new head for an old hat. *Am J Physiol Endocrinol Metab* 2009; **297**: E578-E591 [PMID: 19531645 DOI: 10.1152/ajpendo.00093.2009]

21 **Cline GW**, Petersen KF, Krssak M, Shen J, Hundal RS, Trajanoski Z, Inzucchi S, Dresner A, Rothman DL, Shulman GI. Impaired glucose transport as a cause of decreased insulin-stimulated muscle glycogen synthesis in type 2 diabetes. *N Engl J Med* 1999; **341**: 240-246 [PMID: 10413736 DOI: 10.1056/NEJM199907223410404]

22 **Dresner A**, Laurent D, Marcucci M, Griffin ME, Dufour S, Cline GW, Slezak LA, Andersen DK, Hundal RS, Rothman DL, Petersen KF, Shulman GI. Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinositol 3-kinase activity. *J Clin Invest* 1999; **103**: 253-259 [PMID: 9916137 DOI: 10.1172/JCI5001]

23 **Griffin ME**, Marcucci MJ, Cline GW, Bell K, Barucci N, Lee D, Goodyear LJ, Kraegen EW, White MF, Shulman GI. Free fatty acid-induced insulin resistance is associated with activation of protein kinase C theta and alterations in the insulin signaling cascade. *Diabetes* 1999; **48**: 1270-1274 [PMID: 10342815 DOI: 10.2337/diabetes.48.6.1270]

24 **Roden M**, Price TB, Perseghin G, Petersen KF, Rothman DL, Cline GW, Shulman GI. Mechanism of free fatty acid-induced insulin resistance in humans. *J Clin Invest* 1996; **97**:

2859-2865 [PMID: 8675698 DOI: 10.1172/JCI118742]
25 **Garvey WT**, Maianu L, Zhu JH, Brechtel-Hook G, Wallace P, Baron AD. Evidence for defects in the trafficking and translocation of GLUT4 glucose transporters in skeletal muscle as a cause of human insulin resistance. *J Clin Invest* 1998; **101**: 2377-2386 [PMID: 9616209]
26 **Muoio DM**. Intramuscular triacylglycerol and insulin resistance: guilty as charged or wrongly accused? *Biochim Biophys Acta* 2010; **1801**: 281-288 [PMID: 19958841 DOI: 10.1016/j.bbalip.2009.11.007]
27 **Muoio DM**, Newgard CB. Fatty acid oxidation and insulin action: when less is more. *Diabetes* 2008; **57**: 1455-1456 [PMID: 18511446 DOI: 10.2337/db08-0281]
28 **McGarry JD**. Banting lecture 2001: dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. *Diabetes* 2002; **51**: 7-18 [PMID: 11756317]
29 **Krssak M**, Falk Petersen K, Dresner A, DiPietro L, Vogel SM, Rothman DL, Roden M, Shulman GI. Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a 1H NMR spectroscopy study. *Diabetologia* 1999; **42**: 113-116 [PMID: 10027589]
30 **Kim YB**, Uotani S, Pierroz DD, Flier JS, Kahn BB. In vivo administration of leptin activates signal transduction directly in insulin-sensitive tissues: overlapping but distinct pathways from insulin. *Endocrinology* 2000; **141**: 2328-2339 [PMID: 10875232 DOI: 10.1210/en.141.7.2328]
31 **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/JCI200215001]
32 **Merkel M**, Weinstock PH, Chajek-Shaul T, Radner H, Yin B, Breslow JL, Goldberg IJ. Lipoprotein lipase expression exclusively in liver. A mouse model for metabolism in the neonatal period and during cachexia. *J Clin Invest* 1998; **102**: 893-901 [PMID: 9727057 DOI: 10.1172/JCI2912]
33 **Kim JK**, Fillmore JJ, Chen Y, Yu C, Moore IK, Pypaert M, Lutz EP, Kako Y, Velez-Carrasco W, Goldberg IJ, Breslow JL, Shulman GI. Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin resistance. *Proc Natl Acad Sci USA* 2001; **98**: 7522-7527 [PMID: 11390966 DOI: 10.1073/pnas.121164498]
34 **Fabbrini E**, Magkos F, Mohammed BS, Pietka T, Abumrad NA, Patterson BW, Okunade A, Klein S. Intrahepatic fat, not visceral fat, is linked with metabolic complications of obesity. *Proc Natl Acad Sci USA* 2009; **106**: 15430-15435 [PMID: 19706383 DOI: 10.1073/pnas.0904944106]
35 **Fabbrini E**, Tamboli RA, Magkos F, Marks-Shulman PA, Eckhauser AW, Richards WO, Klein S, Abumrad NN. Surgical removal of omental fat does not improve insulin sensitivity and cardiovascular risk factors in obese adults. *Gastroenterology* 2010; **139**: 448-455 [PMID: 20457158 DOI: 10.1053/j.gastro.2010.04.056]
36 **Goodpaster BH**, He J, Watkins S, Kelley DE. Skeletal muscle lipid content and insulin resistance: evidence for a paradox in endurance-trained athletes. *J Clin Endocrinol Metab* 2001; **86**: 5755-5761 [PMID: 11739435 DOI: 10.1210/jc.86.12.5755]
37 **Kahn BB**, Flier JS. Obesity and insulin resistance. *J Clin Invest* 2000; **106**: 473-481 [PMID: 10953022 DOI: 10.1172/JCI10842]
38 **Hegarty BD**, Furler SM, Ye J, Cooney GJ, Kraegen EW. The role of intramuscular lipid in insulin resistance. *Acta Physiol Scand* 2003; **178**: 373-383 [PMID: 12864742 DOI: 10.1046/j.1365-201X.2003.01162.x]
39 **Ahmadian M**, Duncan RE, Sul HS. The skinny on fat: lipolysis and fatty acid utilization in adipocytes. *Trends Endocrinol Metab* 2009; **20**: 424-428 [PMID: 19796963 DOI: 10.1016/j.tem.2009.06.002]
40 **White MF**. The insulin signalling system and the IRS proteins. *Diabetologia* 1997; **40** Suppl 2: S2-17 [PMID: 9248696 DOI: 10.1007/s001250051387]
41 **Lee YH**, White MF. Insulin receptor substrate proteins and diabetes. *Arch Pharm Res* 2004; **27**: 361-370 [PMID: 15180298 DOI: 10.1007/BF02980074]
42 **Giovannone B**, Scaldaferri ML, Federici M, Porzio O, Lauro D, Fusco A, Sbraccia P, Borboni P, Lauro R, Sesti G. Insulin receptor substrate (IRS) transduction system: distinct and overlapping signaling potential. *Diabetes Metab Res Rev* 2000; **16**: 434-441 [PMID: 11114102 DOI: 10.1002/1520-7560(2000)9999:9999<::AID-DMRR159>3.0.CO;2-8]
43 **Fantin VR**, Wang Q, Lienhard GE, Keller SR. Mice lacking insulin receptor substrate 4 exhibit mild defects in growth, reproduction, and glucose homeostasis. *Am J Physiol Endocrinol Metab* 2000; **278**: E127-E133 [PMID: 10644546]
44 **Liu SC**, Wang Q, Lienhard GE, Keller SR. Insulin receptor substrate 3 is not essential for growth or glucose homeostasis. *J Biol Chem* 1999; **274**: 18093-18099 [PMID: 10364263]
45 **Tsuruzoe K**, Emkey R, Kriauciunas KM, Ueki K, Kahn CR. Insulin receptor substrate 3 (IRS-3) and IRS-4 impair IRS-1- and IRS-2-mediated signaling. *Mol Cell Biol* 2001; **21**: 26-38 [PMID: 11113178 DOI: 10.1074/jbc.274.25.18093]
46 **Vanhaesebroeck B**, Alessi DR. The PI3K-PDK1 connection: more than just a road to PKB. *Biochem J* 2000; **346** Pt 3: 561-576 [PMID: 10698680 DOI: 10.1042/0264-6021:3460561]
47 **Cohen P**, Alessi DR, Cross DA. PDK1, one of the missing links in insulin signal transduction? *FEBS Lett* 1997; **410**: 3-10 [PMID: 9247112 DOI: 10.1016/S0014-5793(97)00490-0]
48 **Litherland GJ**, Hajduch E, Hundal HS. Intracellular signalling mechanisms regulating glucose transport in insulin-sensitive tissues (review). *Mol Membr Biol* 2001; **18**: 195-204 [PMID: 11681786 DOI: 10.1016/S0014-5793(01)02242-6]
49 **Hajduch E**, Litherland GJ, Hundal HS. Protein kinase B (PKB/Akt)--a key regulator of glucose transport? *FEBS Lett* 2001; **492**: 199-203 [PMID: 11257494]
50 **Schultze SM**, Jensen J, Hemmings BA, Tschopp O, Niessen M. Promiscuous affairs of PKB/AKT isoforms in metabolism. *Arch Physiol Biochem* 2011; **117**: 70-77 [PMID: 21214427 DOI: 10.3109/13813455.2010.539236]
51 **Taniguchi CM**, Emanuelli B, Kahn CR. Critical nodes in signalling pathways: insights into insulin action. *Nat Rev Mol Cell Biol* 2006; **7**: 85-96 [PMID: 16493415 DOI: 10.1038/nrm1837]
52 **Sarbassov DD**, Guertin DA, Ali SM, Sabatini DM. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* 2005; **307**: 1098-1101 [PMID: 15718470 DOI: 10.1126/science.1106148]
53 **Jiang ZY**, Zhou QL, Coleman KA, Chouinard M, Boese Q, Czech MP. Insulin signaling through Akt/protein kinase B analyzed by small interfering RNA-mediated gene silencing. *Proc Natl Acad Sci USA* 2003; **100**: 7569-7574 [PMID: 12808134 DOI: 10.1073/pnas.1332633100]
54 **Kim JY**, Hickner RC, Cortright RL, Dohm GL, Houmard JA. Lipid oxidation is reduced in obese human skeletal muscle. *Am J Physiol Endocrinol Metab* 2000; **279**: E1039-E1044 [PMID: 11052958]
55 **Kelley DE**, He J, Menshikova EV, Ritov VB. Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. *Diabetes* 2002; **51**: 2944-2950 [PMID: 12351431 DOI: 10.2337/diabetes.51.10.2944]
56 **Ritov VB**, Menshikova EV, He J, Ferrell RE, Goodpaster BH, Kelley DE. Deficiency of subsarcolemmal mitochondria in obesity and type 2 diabetes. *Diabetes* 2005; **54**: 8-14 [PMID: 15616005 DOI: 10.2337/diabetes.54.1.8]
57 **Heilbronn LK**, Gan SK, Turner N, Campbell LV, Chisholm DJ. Markers of mitochondrial biogenesis and metabolism are lower in overweight and obese insulin-resistant subjects. *J Clin Endocrinol Metab* 2007; **92**: 1467-1473 [PMID: 17244782 DOI: 10.1210/jc.2006-2210]
58 **Koyama K**, Chen G, Lee Y, Unger RH. Tissue triglycerides, insulin resistance, and insulin production: implications for hyperinsulinemia of obesity. *Am J Physiol* 1997; **273**: E708-E713 [PMID: 9357799]

59 **Phillips DI**, Caddy S, Ilic V, Fielding BA, Frayn KN, Borthwick AC, Taylor R. Intramuscular triglyceride and muscle insulin sensitivity: evidence for a relationship in nondiabetic subjects. *Metabolism* 1996; **45**: 947-950 [PMID: 8769349 DOI: 10.1016/S0026-0495(96)90260-7]

60 **Szendroedi J**, Roden M. Ectopic lipids and organ function. *Curr Opin Lipidol* 2009; **20**: 50-56 [PMID: 19133412 DOI: 10.1097/MOL.0b013e328321b3a8]

61 **Thomas EL**, Fitzpatrick JA, Malik SJ, Taylor-Robinson SD, Bell JD. Whole body fat: content and distribution. *Prog Nucl Magn Reson Spectrosc* 2013; **73**: 56-80 [PMID: 23962884 DOI: 10.1016/j.pnmrs.2013.04.001]

62 **Chavez JA**, Summers SA. Characterizing the effects of saturated fatty acids on insulin signaling and ceramide and diacylglycerol accumulation in 3T3-L1 adipocytes and C2C12 myotubes. *Arch Biochem Biophys* 2003; **419**: 101-109 [PMID: 14592453 DOI: 10.1016/j.abb.2003.08.020]

63 **Powell DJ**, Turban S, Gray A, Hajduch E, Hundal HS. Intracellular ceramide synthesis and protein kinase Czeta activation play an essential role in palmitate-induced insulin resistance in rat L6 skeletal muscle cells. *Biochem J* 2004; **382**: 619-629 [PMID: 15193147 DOI: 10.1042/BJ20040139]

64 **Dimopoulos N**, Watson M, Sakamoto K, Hundal HS. Differential effects of palmitate and palmitoleate on insulin action and glucose utilization in rat L6 skeletal muscle cells. *Biochem J* 2006; **399**: 473-481 [PMID: 16822230 DOI: 10.1042/BJ20060244]

65 **Cao H**, Gerhold K, Mayers JR, Wiest MM, Watkins SM, Hotamisligil GS. Identification of a lipokine, a lipid hormone linking adipose tissue to systemic metabolism. *Cell* 2008; **134**: 933-944 [PMID: 18805087 DOI: 10.1016/j.cell.2008.07.048]

66 **Storlien LH**, Kraegen EW, Chisholm DJ, Ford GL, Bruce DG, Pascoe WS. Fish oil prevents insulin resistance induced by high-fat feeding in rats. *Science* 1987; **237**: 885-888 [PMID: 3303333 DOI: 10.1126/science.3303333]

67 **Brose N**, Betz A, Wegmeyer H. Divergent and convergent signaling by the diacylglycerol second messenger pathway in mammals. *Curr Opin Neurobiol* 2004; **14**: 328-340 [PMID: 15194113 DOI: 10.1016/j.conb.2004.05.006]

68 **Kotronen A**, Seppänen-Laakso T, Westerbacka J, Kiviluoto T, Arola J, Ruskeepää AL, Oresic M, Yki-Järvinen H. Hepatic stearoyl-CoA desaturase (SCD)-1 activity and diacylglycerol but not ceramide concentrations are increased in the nonalcoholic human fatty liver. *Diabetes* 2009; **58**: 203-208 [PMID: 18952834 DOI: 10.2337/db08-1074]

69 **Puri P**, Baillie RA, Wiest MM, Mirshahi F, Choudhury J, Cheung O, Sargeant C, Contos MJ, Sanyal AJ. A lipidomic analysis of nonalcoholic fatty liver disease. *Hepatology* 2007; **46**: 1081-1090 [PMID: 17654743 DOI: 10.1002/hep.21763]

70 **Magkos F**, Su X, Bradley D, Fabbrini E, Conte C, Eagon JC, Varela JE, Brunt EM, Patterson BW, Klein S. Intrahepatic diacylglycerol content is associated with hepatic insulin resistance in obese subjects. *Gastroenterology* 2012; **142**: 1444-1446.e2 [PMID: 22425588 DOI: 10.1053/j.gastro.2012.03.003]

71 **Kumashiro N**, Erion DM, Zhang D, Kahn M, Beddow SA, Chu X, Still CD, Gerhard GS, Han X, Dziura J, Petersen KF, Samuel VT, Shulman GI. Cellular mechanism of insulin resistance in nonalcoholic fatty liver disease. *Proc Natl Acad Sci USA* 2011; **108**: 16381-16385 [PMID: 21930939 DOI: 10.1073/pnas.1113359108]

72 **Samuel VT**, Liu ZX, Wang A, Beddow SA, Geisler JG, Kahn M, Zhang XM, Monia BP, Bhanot S, Shulman GI. Inhibition of protein kinase Cepsilon prevents hepatic insulin resistance in nonalcoholic fatty liver disease. *J Clin Invest* 2007; **117**: 739-745 [PMID: 17318260 DOI: 10.1172/JCI130400]

73 **Turban S**, Hajduch E. Protein kinase C isoforms: mediators of reactive lipid metabolites in the development of insulin resistance. *FEBS Lett* 2011; **585**: 269-274 [PMID: 21176778 DOI: 10.1016/j.febslet.2010.12.022]

74 **Jornayvaz FR**, Shulman GI. Diacylglycerol activation of protein kinase Cε and hepatic insulin resistance. *Cell Metab* 2012; **15**: 574-584 [PMID: 22560210 DOI: 10.1016/j.cmet.2012.03.005]

75 **van Herpen NA**, Schrauwen-Hinderling VB. Lipid accumulation in non-adipose tissue and lipotoxicity. *Physiol Behav* 2008; **94**: 231-241 [PMID: 18222498 DOI: 10.1016/j.physbeh.2007.11.049]

76 **Itani SI**, Ruderman NB, Schmieder F, Boden G. Lipid-induced insulin resistance in human muscle is associated with changes in diacylglycerol, protein kinase C, and IkappaB-alpha. *Diabetes* 2002; **51**: 2005-2011 [PMID: 12086926 DOI: 10.2337/diabetes.51.7.2005]

77 **Straczkowski M**, Kowalska I, Baranowski M, Nikolajuk A, Otziomek E, Zabielski P, Adamska A, Blachnio A, Gorski J, Gorska M. Increased skeletal muscle ceramide level in men at risk of developing type 2 diabetes. *Diabetologia* 2007; **50**: 2366-2373 [PMID: 17724577 DOI: 10.1007/s00125-007-0781-2]

78 **Liu L**, Zhang Y, Chen N, Shi X, Tsang B, Yu YH. Upregulation of myocellular DGAT1 augments triglyceride synthesis in skeletal muscle and protects against fat-induced insulin resistance. *J Clin Invest* 2007; **117**: 1679-1689 [PMID: 17510710 DOI: 10.1172/JCI30565]

79 **Chibalin AV**, Leng Y, Vieira E, Krook A, Björnholm M, Long YC, Kotova O, Zhong Z, Sakane F, Steiler T, Nylén C, Wang J, Laakso M, Topham MK, Gilbert M, Wallberg-Henriksson H, Zierath JR. Downregulation of diacylglycerol kinase delta contributes to hyperglycemia-induced insulin resistance. *Cell* 2008; **132**: 375-386 [PMID: 18267070 DOI: 10.1016/j.cell.2007.12.035]

80 **Vistisen B**, Hellgren LI, Vadset T, Scheede-Bergdahl C, Helge JW, Dela F, Stallknecht B. Effect of gender on lipid-induced insulin resistance in obese subjects. *Eur J Endocrinol* 2008; **158**: 61-68 [PMID: 18166818 DOI: 10.1530/EJE-07-0493]

81 **Anastasiou CA**, Kavouras SA, Lentzas Y, Gova A, Sidossis LS, Melidonis A. Diabetes mellitus is associated with increased intramyocellular triglyceride, but not diglyceride, content in obese humans. *Metabolism* 2009; **58**: 1636-1642 [PMID: 19615699 DOI: 10.1016/j.metabol.2009.05.019]

82 **Perreault L**, Bergman BC, Hunerdosse DM, Eckel RH. Altered intramuscular lipid metabolism relates to diminished insulin action in men, but not women, in progression to diabetes. *Obesity (Silver Spring)* 2010; **18**: 2093-2100 [PMID: 20379150 DOI: 10.1038/oby.2010.76]

83 **Amati F**, Dubé JJ, Alvarez-Carnero E, Edreira MM, Chomentowski P, Coen PM, Switzer GE, Bickel PE, Stefanovic-Racic M, Toledo FG, Goodpaster BH. Skeletal muscle triglycerides, diacylglycerols, and ceramides in insulin resistance: another paradox in endurance-trained athletes? *Diabetes* 2011; **60**: 2588-2597 [PMID: 21873552 DOI: 10.2337/db10-1221]

84 **Coen PM**, Hames KC, Leachman EM, DeLany JP, Ritov VB, Menshikova EV, Dubé JJ, Stefanovic-Racic M, Toledo FG, Goodpaster BH. Reduced skeletal muscle oxidative capacity and elevated ceramide but not diacylglycerol content in severe obesity. *Obesity* (Silver Spring) 2013; **21**: 2362-2371 [PMID: 23512750 DOI: 10.1002/oby.20381]

85 **Holland WL**, Summers SA. Sphingolipids, insulin resistance, and metabolic disease: new insights from in vivo manipulation of sphingolipid metabolism. *Endocr Rev* 2008; **29**: 381-402 [PMID: 18451260 DOI: 10.1210/er.2007-0025]

86 **Hannun YA**. The sphingomyelin cycle and the second messenger function of ceramide. *J Biol Chem* 1994; **269**: 3125-3128 [PMID: 8106344]

87 **Adams JM**, Pratipanawatr T, Berria R, Wang E, DeFronzo RA, Sullards MC, Mandarino LJ. Ceramide content is increased in skeletal muscle from obese insulin-resistant humans. *Diabetes* 2004; **53**: 25-31 [PMID: 14693694]

88 **Coen PM**, Dubé JJ, Amati F, Stefanovic-Racic M, Ferrell RE, Toledo FG, Goodpaster BH. Insulin resistance is associated with higher intramyocellular triglycerides in type I but not type II myocytes concomitant with higher ceramide content. *Diabetes*

2010; **59**: 80-88 [PMID: 19833891 DOI: 10.2337/db09-0988]
89 **Lipina C**, Hundal HS. Sphingolipids: agents provocateurs in the pathogenesis of insulin resistance. *Diabetologia* 2011; **54**: 1596-1607 [PMID: 21468641 DOI: 10.1007/s00125-011-2127-3]
90 **Bartke N**, Hannun YA. Bioactive sphingolipids: metabolism and function. *J Lipid Res* 2009; **50** Suppl: S91-S96 [PMID: 19017611 DOI: 10.1194/jlr.R800080-JLR200]
91 **Hannun YA**. Functions of ceramide in coordinating cellular responses to stress. *Science* 1996; **274**: 1855-1859 [PMID: 8943189]
92 **Futerman AH**, Riezman H. The ins and outs of sphingolipid synthesis. *Trends Cell Biol* 2005; **15**: 312-318 [PMID: 15953549 DOI: 10.1016/j.tcb.2005.04.006]
93 **Futerman AH**. Intracellular trafficking of sphingolipids: relationship to biosynthesis. *Biochim Biophys Acta* 2006; **1758**: 1885-1892 [PMID: 16996025 DOI: 10.1016/j.bbamem.2006.08.004]
94 **Mullen TD**, Hannun YA, Obeid LM. Ceramide synthases at the centre of sphingolipid metabolism and biology. *Biochem J* 2012; **441**: 789-802 [PMID: 22248339 DOI: 10.1042/BJ20111626]
95 **Grösch S**, Schiffmann S, Geisslinger G. Chain length-specific properties of ceramides. *Prog Lipid Res* 2012; **51**: 50-62 [PMID: 22133871 DOI: 10.1016/j.plipres.2011.11.001]
96 **Hannun YA**, Obeid LM. Many ceramides. *J Biol Chem* 2011; **286**: 27855-27862 [PMID: 21693702 DOI: 10.1074/jbc.R111.254359]
97 **Turinsky J**, O'Sullivan DM, Bayly BP. 1,2-Diacylglycerol and ceramide levels in insulin-resistant tissues of the rat in vivo. *J Biol Chem* 1990; **265**: 16880-16885 [PMID: 2211599]
98 **Samad F**, Hester KD, Yang G, Hannun YA, Bielawski J. Altered adipose and plasma sphingolipid metabolism in obesity: a potential mechanism for cardiovascular and metabolic risk. *Diabetes* 2006; **55**: 2579-2587 [PMID: 16936207 DOI: 10.2337/db06-0330]
99 **Schmitz-Peiffer C**, Craig DL, Biden TJ. Ceramide generation is sufficient to account for the inhibition of the insulin-stimulated PKB pathway in C2C12 skeletal muscle cells pretreated with palmitate. *J Biol Chem* 1999; **274**: 24202-24210 [PMID: 10446195 DOI: 10.1074/jbc.274.34.24202]
100 **Hajduch E**, Balendran A, Batty IH, Litherland GJ, Blair AS, Downes CP, Hundal HS. Ceramide impairs the insulin-dependent membrane recruitment of protein kinase B leading to a loss in downstream signalling in L6 skeletal muscle cells. *Diabetologia* 2001; **44**: 173-183 [PMID: 11270673 DOI: 10.1007/s001250051596]
101 **Summers SA**, Garza LA, Zhou H, Birnbaum MJ. Regulation of insulin-stimulated glucose transporter GLUT4 translocation and Akt kinase activity by ceramide. *Mol Cell Biol* 1998; **18**: 5457-5464 [PMID: 9710629]
102 **Straczkowski M**, Kowalska I, Nikolajuk A, Dzienis-Straczkowska S, Kinalska I, Baranowski M, Zendzian-Piotrowska M, Brzezinska Z, Gorski J. Relationship between insulin sensitivity and sphingomyelin signaling pathway in human skeletal muscle. *Diabetes* 2004; **53**: 1215-1221 [PMID: 15111489 DOI: 10.2337/diabetes.53.5.1215]
103 **Dubé JJ**, Amati F, Stefanovic-Racic M, Toledo FG, Sauers SE, Goodpaster BH. Exercise-induced alterations in intramyocellular lipids and insulin resistance: the athlete's paradox revisited. *Am J Physiol Endocrinol Metab* 2008; **294**: E882-E888 [PMID: 18319352 DOI: 10.1152/ajpendo.00769.2007]
104 **Blachnio-Zabielska AU**, Koutsari C, Tchkonia T, Jensen MD. Sphingolipid content of human adipose tissue: relationship to adiponectin and insulin resistance. *Obesity* (Silver Spring) 2012; **20**: 2341-2347 [PMID: 22677645 DOI: 10.1038/oby.2012.126]
105 **Błachnio-Zabielska AU**, Pułka M, Baranowski M, Nikołajuk A, Zabielski P, Górska M, Górski J. Ceramide metabolism is affected by obesity and diabetes in human adipose tissue. *J Cell Physiol* 2012; **227**: 550-557 [PMID: 21437908 DOI: 10.1002/jcp.22745]
106 **Memon RA**, Holleran WM, Moser AH, Seki T, Uchida Y, Fuller J, Shigenaga JK, Grunfeld C, Feingold KR. Endotoxin and cytokines increase hepatic sphingolipid biosynthesis and produce lipoproteins enriched in ceramides and sphingomyelin. *Arterioscler Thromb Vasc Biol* 1998; **18**: 1257-1265 [PMID: 9714132]
107 **Longato L**, Tong M, Wands JR, de la Monte SM. High fat diet induced hepatic steatosis and insulin resistance: Role of dysregulated ceramide metabolism. *Hepatol Res* 2012; **42**: 412-427 [PMID: 22176347 DOI: 10.1111/j.1872-034X.2011.00934.x]
108 **Watt MJ**, Barnett AC, Bruce CR, Schenk S, Horowitz JF, Hoy AJ. Regulation of plasma ceramide levels with fatty acid oversupply: evidence that the liver detects and secretes de novo synthesised ceramide. *Diabetologia* 2012; **55**: 2741-2746 [PMID: 22854889 DOI: 10.1007/s00125-012-2649-3]
109 **Lightle S**, Tosheva R, Lee A, Queen-Baker J, Boyanovsky B, Shedlofsky S, Nikolova-Karakashian M. Elevation of ceramide in serum lipoproteins during acute phase response in humans and mice: role of serine-palmitoyl transferase. *Arch Biochem Biophys* 2003; **419**: 120-128 [PMID: 14592455 DOI: 10.1016/j.abb.2003.08.031]
110 **Merrill AH**, Lingrell S, Wang E, Nikolova-Karakashian M, Vales TR, Vance DE. Sphingolipid biosynthesis de novo by rat hepatocytes in culture. Ceramide and sphingomyelin are associated with, but not required for, very low density lipoprotein secretion. *J Biol Chem* 1995; **270**: 13834-13841 [PMID: 7775441]
111 **Kluepfel D**, Bagli J, Baker H, Charest MP, Kudelski A. Myriocin, a new antifungal antibiotic from Myriococcum albomyces. *J Antibiot* (Tokyo) 1972; **25**: 109-115 [PMID: 5034807]
112 **Ussher JR**, Koves TR, Cadete VJ, Zhang L, Jaswal JS, Swyrd SJ, Lopaschuk DG, Proctor SD, Keung W, Muoio DM, Lopaschuk GD. Inhibition of de novo ceramide synthesis reverses diet-induced insulin resistance and enhances whole-body oxygen consumption. *Diabetes* 2010; **59**: 2453-2464 [PMID: 20522596 DOI: 10.2337/db09-1293]
113 **Holland WL**, Brozinick JT, Wang LP, Hawkins ED, Sargent KM, Liu Y, Narra K, Hoehn KL, Knotts TA, Siesky A, Nelson DH, Karathanasis SK, Fontenot GK, Birnbaum MJ, Summers SA. Inhibition of ceramide synthesis ameliorates glucocorticoid-, saturated-fat-, and obesity-induced insulin resistance. *Cell Metab* 2007; **5**: 167-179 [PMID: 17339025 DOI: 10.1016/j.cmet.2007.01.002]
114 **Yang G**, Badeanlou L, Bielawski J, Roberts AJ, Hannun YA, Samad F. Central role of ceramide biosynthesis in body weight regulation, energy metabolism, and the metabolic syndrome. *Am J Physiol Endocrinol Metab* 2009; **297**: E211-E224 [PMID: 19435851 DOI: 10.1152/ajpendo.91014.2008]
115 **Bikman BT**, Guan Y, Shui G, Siddique MM, Holland WL, Kim JY, Fabriàs G, Wenk MR, Summers SA. Fenretinide prevents lipid-induced insulin resistance by blocking ceramide biosynthesis. *J Biol Chem* 2012; **287**: 17426-17437 [PMID: 22474281 DOI: 10.1074/jbc.M112.359950]
116 **Kurek K**, Piotrowska DM, Wiesiołek-Kurek P, Lukaszuk B, Chabowski A, Górski J, Zendzian-Piotrowska M. Inhibition of ceramide de novo synthesis reduces liver lipid accumulation in rats with nonalcoholic fatty liver disease. *Liver Int* 2013; Epub ahead of print [PMID: 24106929 DOI: 10.1111/liv.12331]
117 **Le Stunff H**, Milstien S, Spiegel S. Generation and metabolism of bioactive sphingosine-1-phosphate. *J Cell Biochem* 2004; **92**: 882-899 [PMID: 15258913 DOI: 10.1002/h=jcb.20097]
118 **Maceyka M**, Harikumar KB, Milstien S, Spiegel S. Sphingosine-1-phosphate signaling and its role in disease. *Trends Cell Biol* 2012; **22**: 50-60 [PMID: 22001186 DOI: 10.1016/j.tcb.2011.09.003]
119 **Ma MM**, Chen JL, Wang GG, Wang H, Lu Y, Li JF, Yi J, Yuan YJ, Zhang QW, Mi J, Wang LSh, Duan HF, Wu CT. Sphingosine kinase 1 participates in insulin signalling and regulates glucose metabolism and homeostasis in KK/Ay diabetic mice. *Diabetologia* 2007; **50**: 891-900 [PMID: 17265031 DOI: 10.1007/s00125-006-0589-5]
120 **Rapizzi E**, Taddei ML, Fiaschi T, Donati C, Bruni P, Chia-

rugi P. Sphingosine 1-phosphate increases glucose uptake through trans-activation of insulin receptor. *Cell Mol Life Sci* 2009; **66**: 3207-3218 [PMID: 19662499 DOI: 10.1007/s00018-009-0106-3]

121 **Morales-Ruiz M**, Lee MJ, Zöllner S, Gratton JP, Scotland R, Shiojima I, Walsh K, Hla T, Sessa WC. Sphingosine 1-phosphate activates Akt, nitric oxide production, and chemotaxis through a Gi protein/phosphoinositide 3-kinase pathway in endothelial cells. *J Biol Chem* 2001; **276**: 19672-19677 [PMID: 11278592 DOI: 10.1074/jbc.M009993200]

122 **Bruce CR**, Risis S, Babb JR, Yang C, Kowalski GM, Selathurai A, Lee-Young RS, Weir JM, Yoshioka K, Takuwa Y, Meikle PJ, Pitson SM, Febbraio MA. Overexpression of sphingosine kinase 1 prevents ceramide accumulation and ameliorates muscle insulin resistance in high-fat diet-fed mice. *Diabetes* 2012; **61**: 3148-3155 [PMID: 22961081 DOI: 10.2337/db12-0029]

123 **Lahiri S**, Park H, Laviad EL, Lu X, Bittman R, Futerman AH. Ceramide synthesis is modulated by the sphingosine analog FTY720 via a mixture of uncompetitive and noncompetitive inhibition in an Acyl-CoA chain length-dependent manner. *J Biol Chem* 2009; **284**: 16090-16098 [PMID: 19357080 DOI: 10.1074/jbc.M807438200]

124 **Bruce CR**, Risis S, Babb JR, Yang C, Lee-Young RS, Henstridge DC, Febbraio MA. The sphingosine-1-phosphate analog FTY720 reduces muscle ceramide content and improves glucose tolerance in high fat-fed male mice. *Endocrinology* 2013; **154**: 65-76 [PMID: 23183172 DOI: 10.1210/en.2012-1847]

125 **Chavez JA**, Holland WL, Bär J, Sandhoff K, Summers SA. Acid ceramidase overexpression prevents the inhibitory effects of saturated fatty acids on insulin signaling. *J Biol Chem* 2005; **280**: 20148-20153 [PMID: 15774472 DOI: 10.1074/jbc.M412769200]

126 **Kanety H**, Hemi R, Papa MZ, Karasik A. Sphingomyelinase and ceramide suppress insulin-induced tyrosine phosphorylation of the insulin receptor substrate-1. *J Biol Chem* 1996; **271**: 9895-9897 [PMID: 8626623]

127 **Zundel W**, Giaccia A. Inhibition of the anti-apoptotic PI(3)K/Akt/Bad pathway by stress. *Genes Dev* 1998; **12**: 1941-1946 [PMID: 9649498 DOI: 10.1101/gad.12.13.1941]

128 **Stratford S**, Hoehn KL, Liu F, Summers SA. Regulation of insulin action by ceramide: dual mechanisms linking ceramide accumulation to the inhibition of Akt/protein kinase B. *J Biol Chem* 2004; **279**: 36608-36615 [PMID: 15220355 DOI: 10.1074/jbc.M406499200]

129 **Teruel T**, Hernandez R, Lorenzo M. Ceramide mediates insulin resistance by tumor necrosis factor-alpha in brown adipocytes by maintaining Akt in an inactive dephosphorylated state. *Diabetes* 2001; **50**: 2563-2571 [PMID: 11679435 DOI: 10.2337/diabetes.50.11.2563]

130 **Bourbon NA**, Sandirasegarane L, Kester M. Ceramide-induced inhibition of Akt is mediated through protein kinase Czeta: implications for growth arrest. *J Biol Chem* 2002; **277**: 3286-3292 [PMID: 11723139 DOI: 10.1074/jbc.M110541200]

131 **Martin D**, Salinas M, Fujita N, Tsuruo T, Cuadrado A. Ceramide and reactive oxygen species generated by H2O2 induce caspase-3-independent degradation of Akt/protein kinase B. *J Biol Chem* 2002; **277**: 42943-42952 [PMID: 12213802 DOI: 10.1074/jbc.M201070200]

132 **Blouin CM**, Prado C, Takane KK, Lasnier F, Garcia-Ocana A, Ferré P, Dugail I, Hajduch E. Plasma membrane subdomain compartmentalization contributes to distinct mechanisms of ceramide action on insulin signaling. *Diabetes* 2010; **59**: 600-610 [PMID: 19959757 DOI: 10.2337/db09-0897]

133 **Janssens V**, Goris J. Protein phosphatase 2A: a highly regulated family of serine/threonine phosphatases implicated in cell growth and signalling. *Biochem J* 2001; **353**: 417-439 [PMID: 11171037 DOI: 10.1042/0264-6021:3530417]

134 **Srinivasan M**, Begum N. Regulation of protein phosphatase 1 and 2A activities by insulin during myogenesis in rat skeletal muscle cells in culture. *J Biol Chem* 1994; **269**: 12514-12520 [PMID: 8175660]

135 **Cazzolli R**, Carpenter L, Biden TJ, Schmitz-Peiffer C. A role for protein phosphatase 2A-like activity, but not atypical protein kinase Czeta, in the inhibition of protein kinase B/Akt and glycogen synthesis by palmitate. *Diabetes* 2001; **50**: 2210-2218 [PMID: 11574400 DOI: 10.2337/diabetes.50.10.2210]

136 **Salinas M**, López-Valdaliso R, Martín D, Alvarez A, Cuadrado A. Inhibition of PKB/Akt1 by C2-ceramide involves activation of ceramide-activated protein phosphatase in PC12 cells. *Mol Cell Neurosci* 2000; **15**: 156-169 [PMID: 10673324 DOI: 10.1006/mcne.1999.0813]

137 **Cho US**, Morrone S, Sablina AA, Arroyo JD, Hahn WC, Xu W. Structural basis of PP2A inhibition by small t antigen. *PLoS Biol* 2007; **5**: e202 [PMID: 17608567 DOI: 10.1371/journal.pbio.0050202]

138 **Konishi H**, Kuroda S, Kikkawa U. The pleckstrin homology domain of RAC protein kinase associates with the regulatory domain of protein kinase C zeta. *Biochem Biophys Res Commun* 1994; **205**: 1770-1775 [PMID: 7811263 DOI: 10.1006/bbrc.1994.2874]

139 **Doornbos RP**, Theelen M, van der Hoeven PC, van Blitterswijk WJ, Verkleij AJ, van Bergen en Henegouwen PM. Protein kinase Czeta is a negative regulator of protein kinase B activity. *J Biol Chem* 1999; **274**: 8589-8596 [PMID: 10085094 DOI: 10.1074/jbc.274.13.8589]

140 **Mao M**, Fang X, Lu Y, Lapushin R, Bast RC, Mills GB. Inhibition of growth-factor-induced phosphorylation and activation of protein kinase B/Akt by atypical protein kinase C in breast cancer cells. *Biochem J* 2000; **352** Pt 2: 475-482 [PMID: 11085941 DOI: 10.1042/0264-6021:3520475]

141 **Powell DJ**, Hajduch E, Kular G, Hundal HS. Ceramide disables 3-phosphoinositide binding to the pleckstrin homology domain of protein kinase B (PKB)/Akt by a PKCzeta-dependent mechanism. *Mol Cell Biol* 2003; **23**: 7794-7808 [PMID: 14560023 DOI: 10.1128/MCB.23.21.7794-7808.2003]

142 **Fox TE**, Houck KL, O'Neill SM, Nagarajan M, Stover TC, Pomianowski PT, Unal O, Yun JK, Naides SJ, Kester M. Ceramide recruits and activates protein kinase C zeta (PKC zeta) within structured membrane microdomains. *J Biol Chem* 2007; **282**: 12450-12457 [PMID: 17308302 DOI: 10.1074/jbc.M700082200]

143 **Hajduch E**, Bourron O. Type 2 diabetes: ceramides as a therapeutic rarget? *Clin Lipidol* 2013; **8**: 607-609 [DOI: 10.2217/clp.13.65]

144 **Reyland ME**. Protein kinase C isoforms: Multi-functional regulators of cell life and death. *Front Biosci* (Landmark Ed) 2009; **14**: 2386-2399 [PMID: 19273207 DOI: 10.2741/3385]

145 **Lambrecht C**, Haesen D, Sents W, Ivanova E, Janssens V. Structure, regulation, and pharmacological modulation of PP2A phosphatases. *Methods Mol Biol* 2013; **1053**: 283-305 [PMID: 23860660 DOI: 10.1007/978-1-62703-562-0_17]

146 **Véret J**, Coant N, Berdyshev EV, Skobeleva A, Therville N, Bailbé D, Gorshkova I, Natarajan V, Portha B, Le Stunff H. Ceramide synthase 4 and de novo production of ceramides with specific N-acyl chain lengths are involved in glucolipotoxicity-induced apoptosis of INS-1 β-cells. *Biochem J* 2011; **438**: 177-189 [PMID: 21592087 DOI: 10.1042/BJ20101386]

147 **Cinar R**, Godlewski G, Liu J, Tam J, Jourdan T, Mukhopadhyay B, Harvey-White J, Kunos G. Hepatic cannabinoid-1 receptors mediate diet-induced insulin resistance by increasing de novo synthesis of long-chain ceramides. *Hepatology* 2014; **59**: 143-153 [PMID: 23832510 DOI: 10.1002/hep.26606]

148 **Park JW**, Park WJ, Kuperman Y, Boura-Halfon S, Pewzner-Jung Y, Futerman AH. Ablation of very long acyl chain sphingolipids causes hepatic insulin resistance in mice due to altered detergent-resistant membranes. *Hepatology* 2013; **57**: 525-532 [PMID: 22911490 DOI: 10.1002/hep.26015]

149 **Bajpeyi S**, Myrland CK, Covington JD, Obanda D, Cefalu WT, Smith SR, Rustan AC, Ravussin E. Lipid in skeletal muscle myotubes is associated to the donors' insulin sensitivity and physical activity phenotypes. *Obesity* (Silver Spring)

2014; **22**: 426-434 [PMID: 23818429 DOI: 10.1002/oby.20556]
150 **Frangioudakis G**, Diakanastasis B, Liao BQ, Saville JT, Hoffman NJ, Mitchell TW, Schmitz-Peiffer C. Ceramide accumulation in L6 skeletal muscle cells due to increased activity of ceramide synthase isoforms has opposing effects on insulin action to those caused by palmitate treatment. *Diabetologia* 2013; **56**: 2697-2701 [PMID: 23989724 DOI: 10.1007/s00125-013-3035-5]

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TOPIC HIGHLIGHT

WJD 5th Anniversary Special Issues (1): Insulin

Impact of hypoglycemic agents on myocardial ischemic preconditioning

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Abstract

Murry *et al* in 1986 discovered the intrinsic mechanism of profound protection called ischemic preconditioning. The complex cellular signaling cascades underlying this phenomenon remain controversial and are only partially understood. However, evidence suggests that adenosine, released during the initial ischemic insult, activates a variety of G protein-coupled agonists, such as opioids, bradykinin, and catecholamines, resulting in the activation of protein kinases, especially protein kinase C (PKC). This leads to the translocation of PKC from the cytoplasm to the sarcolemma, where it stimulates the opening of the ATP-sensitive K^+ channel, which confers resistance to ischemia. It is known that a range of different hypoglycemic agents that activate the same signaling cascades at various cellular levels can interfere with protection from ischemic preconditioning. This review examines the effects of several hypoglycemic agents on myocardial ischemic preconditioning in animal studies and clinical trials.

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Key words: Ischemic preconditioning; Myocardial ischemia; Coronary artery disease; Hypoglycemic agents; Diabetes mellitus

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INTRODUCTION

In the last 3 decades, the prevalence of diabetes mellitus in adults 18 years and older has increased 2-fold[1]. Approximately 50%-60% of patients with diabetes die from cardiovascular disease (CVD)[2]. Among various CVDs, acute myocardial infarction (AMI) has a high rate of mortality, and infarct size is a primary determinant of prognosis in these patients[3-5]. Furthermore, patients with diabetes are more likely than patients without diabetes to develop heart failure after AMI[6]. Thus, the development of new cardioprotective strategies capable of protecting the myocardium are imperative in order to improve clinical outcomes in diabetic patients with coronary heart disease. Moreover, hyperglycemia is an important risk factor for coronary artery disease and death; however, the use of some medications to achieve glycemic control is controversial, as their use has not consistently been shown to reduce mortality. The University Group Diabetes Program (UGDP) in 1970 showed that the administration of tolbutamide, a first-generation sulfonylurea, may increase the risk of cardiovascular death[7].

As a cardioprotective strategy, ischemic preconditioning (IPC) has received much attention for its powerful infarct size-limiting effect. This intrinsic mechanism of profound protection was suggested by Murry *et al*[8] in 1986 who found in a canine model that 4 consecutive periods of coronary occlusion of 5 min were able to reduce

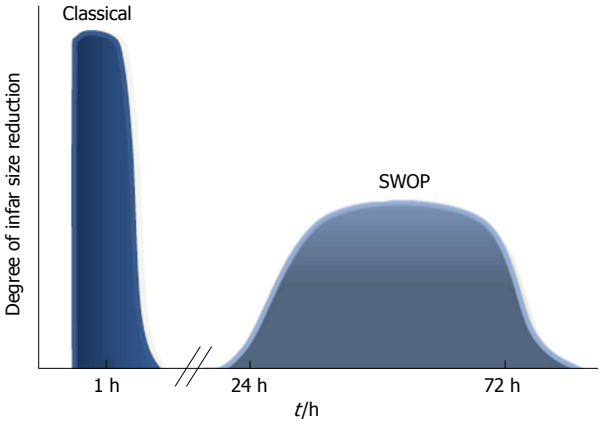

Figure 1 Diagrammatic representation of the temporal nature of the 2 windows of preconditioning (adapted from Baxter *et al*[9]). SWOP: Second window of protection.

the infarct size by as much as 75%, after induction by a subsequent period of occlusion for 40 min. For the first time, it was demonstrated that limitation of infarct size was theoretically possible.

IPC causes 2 phases of protection: "early" or "first window" and "second window of protection" (SWOP). The first window protects the heart for about 2 h and then wanes; the SWOP appears 24 h after the initiation of the IPC protocol and can last for 3 d (Figure 1)[9].

Although IPC was initially referred to as the ability of short periods of ischemia to limit infarct size, some investigators extended this definition to include a beneficial effect on reperfusion-induced arrhythmias[10] and on myocardial stunning[11].

Experimental findings on IPC cannot be directly extrapolated to humans, because of obvious ethical restrictions and because its mechanisms are different from those of other animal species. IPC in human hearts has been demonstrated by results of *in vitro* experiments using human ventricular myocytes[12] and atrial trabeculae[13]. In addition, surrogate clinical endpoints have also been used, including contractile function, electrocardiographic ischemic changes, or biochemical evidence of cell damage.

CELLULAR MECHANISMS OF CLASSICAL PRECONDITIONING

The cellular mechanisms that confer resistance to ischemia have been extensively studied. However, these pathways remain controversial and are only partially understood[14,15]. It has been proposed that endogenous adenosine released during the brief ischemia of the IPC protocol enhances the release of G-protein coupled receptor (GPCR) agonists, such as opioids, adenosine, bradykinin, or catecholamines[16-18]. These GPCR agonists appear to work simultaneously and in parallel to provide redundancy to the preconditioning stimulus. Although these 3 receptors trigger signaling through divergent pathways, this signaling activates prosurvival kinase or reperfusion injury salvage kinase paths, including phosphatidylinositol 3-kinase, protein kinase B, and protein kinase C[14,15]. In turn, it leads to the translocation of protein kinases from the cytoplasm to sarcolemmal receptors[19] and mitochondrial membranes[20], where it phosphorylates a substrate protein, the ATP-sensitive K^+ (KATP) channel[21]. Marinovic *et al*[22] demonstrated in mouse cardiac myocyte cells that the opening of the sarcolemmal KATP channels plays an important role in the prevention of cardiomyocyte apoptosis during metabolic stress, and may interact with mitochondrial channels. Thus, opening of KATP channels are strongly involved in the protection provided by preconditioning[23-26].

Due to the growing knowledge about the cellular pathways of this important protective mechanism, we must consider whether IPC can be applied as a cardioprotective therapy in ischemic heart disease patients.

PHARMACOLOGICAL INTERACTIONS

Pharmacological agents have the capacity to either interfere with signaling or trigger protection. The use of agents capable of mimicking the protective effects of preconditioning, besides brief ischemia, may offer a more benign approach for eliciting cardioprotection. Agents commonly used in coronary disease may interfere with the protection of IPC pathways. Penson *et al*[27] demonstrated in rat-isolated atria and ventricles that activation of beta-adrenoceptors mimics preconditioning. However, β-adrenoceptor blockers impair cardioprotection in animals[28]. Other agents such as Ca^{2+} channel blockers[29] and nonsteroidal anti-inflammatories may interfere with protection by IPC pathways[30,31]. Liu *et al*[16] reported that an adenosine receptor antagonist could block IPC protection and that adenosine or the A1-selective agonist adenosine, instead of brief ischemia, could duplicate IPC protection. Other potential candidates currently in clinical use include nicorandil or diazoxide[32,33]. These drugs have been shown to open KATP channels in ischemic cardiomyocytes, and might act as pharmacological imitators of the preconditioning phenomenon.

HYPOGLYCEMIC DRUGS AND IPC

Hyperglycemia is an important risk factor for coronary artery disease and death. However, the use of some hypoglycemic medications is controversial, because they have not been shown to reduce mortality. Indeed, physicians face challenges regarding the use of new agents in patients with diabetes who are at high cardiovascular risk. Several factors contribute to this concern, and among these is IPC. As described above, the UGDP raised concerns that the administration of tolbutamide may increase the risk of cardiovascular death, but this result remained unexplained until data were reported suggesting deleterious effects of some sulfonylureas (glyburide), specifically in the mechanisms of IPC[23,24].

Insulin secretagogues stimulate insulin secretion by the shutdown of the KATP channel in pancreatic β

cells[34]. KATP channels are composed of 2 types of subunits, inwardly rectifying K^+ channels (Kir6.x) and sulfonylurea receptors (SURx), arranged as tetradimeric complexes (Kir6.x/SURx)[35]. Closure of the KATP channel results in membrane depolarization and influx of calcium (Ca^{2+}) into the β cell. The increase in intracellular Ca^{2+} causes release of insulin from β cell secretory granules. KATP channels are also abundant in both cardiomyocytes[36,37] and arterial smooth muscle cells[38].

The β cell and cardiac muscle KATP channels have been shown to possess a common pore-forming subunit (Kir6.2) but different sulfonylurea receptor subunits (SUR1 and SUR2A, respectively). Although the roles of KATP channel in extrapancreatic tissues are less well characterized, it is likely that they open in response to metabolic stress, such as during cardiac ischemia[39]. Thus, the ideal sulfonylurea for treatment of type 2 diabetes would be one that interacts only with the β cell KATP channel.

EFFECT OF SULFONYLUREAS ON IPC

There is concern about the effect of sulfonylureas on preconditioning protection. Unfortunately, little is known about the ability of the clinically used insulin secretagogues to interfere with IPC. To evaluate studies on the effects of sulfonylureas on IPC, it is important to assess their selectivity for SUR receptor subtypes. These drugs have a range of affinities for KATP channels with different SUR isoform composition, resulting in different abilities to stimulate the KATP channel activity. Tolbutamide has a high affinity for SUR 1 receptors in β cells, but a very low affinity for SUR 2A receptors in the myocardium[40,41]. Glibenclamide (glyburide) inhibits cardiac as well as pancreatic receptors with high affinity[42,43]. Glimepiride has affinity for pancreatic and cardiac SUR comparable to glibenclamide, thereby, does not differentiate between B cells, cardiac muscle, or smooth muscle KATP channels[43,44]. In contrast, preliminary studies reported that glimepiride had less cardiovascular activity than glibenclamide had[45-48]. Several reasons seem to correlate with this finding and, among them, highlight the difference in selectivity for SUR between *in vitro* and *in vivo* studies, and different effects of doses utilized in most studies and in treatment of patients with type 2 diabetes mellitus. In addition, gliclazide, a second generation sulfonylurea, is distinguished by having a higher selectivity for pancreatic SUR receptors[43,49].

Numerous studies using animal models support the hypothesis that IPC is impaired by glibenclamide[23,47,50,51]. Studies using human hearts analyzed IPC in isolated human atrial muscle trabeculae, obtained from type 2 diabetic patients treated with sulfonylureas before coronary artery surgery, and noted that IPC was abolished in patients receiving sulfonylureas[52]. Tomai *et al*[53] evaluated IPC in 20 patients pretreated with either glibenclamide or placebo. They recorded ST-segment changes on ECGs during 2 subsequent episodes of intracoronary balloon inflation. They concluded that human IPC during brief repeated coronary occlusions was completely abolished by pretreatment with glibenclamide. Similar results were shown when the effects of glibenclamide and glimepiride were compared during balloon inflation in percutaneous transluminal coronary angioplasty[45,54].

Tomai *et al*[55] investigated the effects of glibenclamide on the "warm up phenomenon", which is a clinical model of IPC. It refers to an increased tolerance to myocardial ischemia during the second of 2 consecutive exercise tests. In this study, glibenclamide abolished the improvement in ischemic threshold during the second exercise test, compared with placebo[55]. Ovünç[56], in a similar study reported concordant results and suggested that glibenclamide should be used with caution in patients with coronary heart disease and diabetes mellitus, because this agent leads to a decrease in ischemic threshold and exercise capacity. Ferreira *et al*[57], in a study in which IPC was evaluated by 2 consecutive exercise tests, also investigated the effects of chronic treatment with glibenclamide. Forty patients with angina pectoris were allocated into 3 groups: 20 nondiabetic patients, 10 diabetic patients receiving treatment with glibenclamide for at least 6 mo, and 10 diabetic patients receiving other treatments. All patients underwent 2 consecutive exercise tests. The results suggested that IPC protection was blocked in diabetic patients exposed to long-term treatment with glibenclamide. In a recent study, Bilinska *et al*[58] evaluated 64 men, 17 nondiabetic and 47 diabetic, aged 54 ± 5 years. Diabetic patients were allocated into 3 groups: one treated with glibenclamide, one with gliclazide, and the other with diet. All patients performed 2 consecutive exercise tests, with 30 min between them. The authors compared the improvement in ischemic parameters among these groups of patients and concluded that the warm-up effect was preserved in diabetic patients treated with diet, partially preserved in patients treated with gliclazide, and abolished in patients treated with glibenclamide. In contrast, other studies reported no effect of treatment with glibenclamide on the electrocardiographic shifts of the ST-segment during consecutive exercise tests[59,60].

In summary, most studies with glibenclamide (glyburide) reported deleterious effects on IPC, suggesting caution with the use of this agent in patients at high risk for myocardial ischemia.

In animal studies, glimepiride treatment facilitated the cardioprotective effect elicited by IPC[47,48,61-63]. Indeed, data from clinical studies is of great interest. Experimental findings on IPC cannot be directly extrapolated to humans, because in humans its mechanisms are different from those in other animal species. Thus, Klepzig *et al*[45] compared the effects of glibenclamide, glimepiride, and placebo administration on ST-segment shifts during balloon inflation in percutaneous transluminal coronary angioplasty. They concluded that IPC was maintained after glimepiride administration and prevented after glibenclamide. Lee *et al*[46], studied the impact of glibenclamide or glimepiride administration on cardioprotective effects in patients with and without diabetes undergoing coronary angioplasty. The results demonstrated that the

changes in the ST-segment and metabolic parameters were more severe after pretreatment with glibenclamide than with glimepiride, in patients with and without type 2 diabetes.

Only a few studies[45,46] have used IPC protocols in humans to evaluate the effect of glimepiride. To date, these trials have revealed beneficial effects on cardioprotective mechanisms.

In isolated Langendorff perfused rat hearts, the infarct sizes were smaller in the group treated with gliclazide compared with the group treated with glibenclamide. However, the glimepiride group had a smaller infarct size than the gliclazide group[48]. In an *in-vivo* rat study, Maddock *et al*[51] compared the effects of glibenclamide and gliclazide on IPC and nicorandil-induced protection. The IPC protocol consisted of 2 cycles of 5 min of regional ischemia/reperfusion preceding prolonged ischemia. Gliclazide had no adverse effects on IPC or on nicorandil-induced protection. Loubani *et al*[64] assessed the dose-response effect of gliclazide and glibenclamide on IPC. Different doses of glibenclamide and gliclazide were added for 10 min prior to implementation of the IPC protocol. The cardioprotection was abolished by gliclazide only at supratherapeutic concentrations, while glibenclamide prevented IPC at all concentrations.

Bilinska *et al*[58] evaluated the effects of diet, glibenclamide, or gliclazide on the warm-up phenomenon in type 2 diabetic patients with stable angina. They concluded that the warm-up effect was partially preserved in the gliclazide-treated and abolished in the glibenclamide-treated group.

The analysis of the reported data described above suggests that gliclazide does not induce potentially harmful IPC effects.

EFFECT OF GLINIDES ON IPC

The drugs from the glinide class are characterized as insulinotropic agents with a rapid onset and short duration of action. Although glinides do not have a sulfonylurea structure, their role as an insulin secretagogue occurs by binding to the Kir6.2/SUR1 complex, which leads to the closure of KATP channels.

Glinides non-selectively inhibit the pancreatic, myocardial, and non-vascular smooth muscle KATP channels[65]. For these reasons, the selectivity of glinides for the pancreatic compared with the cardiovascular KATP channels has relevance for IPC. Unfortunately, little is known about the ability of the clinically used glinides to interfere with IPC. An original study conducted in our service[66], evaluated the effect of repaglinide on the warm-up phenomenon. Forty-two patients with type 2 diabetes mellitus and coronary artery disease underwent 2 consecutive treadmill exercise tests. After 7 d of receiving repaglinide, 83% of patients no longer had myocardial IPC.

Due to the great difference of *in vitro* selectivity ratios of repaglinide and other drugs in the glinide class (mitiglinide and nateglinide)[43,65], clinical studies assessing the effect of glinides on type 2 diabetic patients with coronary artery disease would be of great interest for both therapeutic and scientific reasons.

EFFECT OF INCRETINS ON IPC

Incretins are gut-derived peptides secreted in response to meals, specifically in the presence and absorption of nutrients in the intestinal lumen. The major incretins are glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide. Incretin is mainly represented by GLP-1. The half-life of GLP-1(7-36) in circulation is very brief (1 to 2 min), as it is rapidly degraded by the enzyme dipeptidyl peptidase-Ⅳ (DPP-Ⅳ) to the metabolite GLP-1(9-36), which does not act on the GLP-1 receptor. GLP-1 receptors are expressed in pancreatic islet cells and in the kidney, lung, brain, gastrointestinal tract, and heart[67]. The incretin modulator class includes the GLP-1 analogues or mimetics, which are functional agonists of the GLP-1 receptor. In addition, oral inhibitors of DPP-Ⅳ, in essence, increase the plasma concentrations of the biologically active form of endogenously secreted incretins[68]. Bose *et al*[69] observed in an isolated rat heart model that GLP-1(7-36) is protective against myocardial ischemia-reperfusion injury when given either as a preconditioning mimetic or at reperfusion. Although several investigators have reported the cardioprotective effect of GLP-1, there is a lack of studies about its effects on IPC. Our research group compared the actions of the DPP-Ⅳ inhibitor (vildagliptin) and repaglinide using an IPC protocol. The results showed that vildagliptin preserved IPC in 72% of 54 patients, while repaglinide maintained the cardioprotective response in only 17% of 42 patients[70]. Our group demonstrated 2 effects of hypoglycemic drugs on IPC. These findings support the importance of identifying underlying mechanisms of endogenous myocardial protection to improve the protective effect of pharmacological therapy (Table 1).

EFFECTS OF GLITAZONES ON IPC

The glitazones or thiazolidinediones offer the first therapeutic option specifically directed at reversing the basic problem of type 2 diabetes, which is resistance to insulin. These drugs act on tissues such as liver and skeletal muscle, sensitizing them to insulin action, and thereby increasing glucose uptake and decreasing its hepatic output. The oldest and best-studied glitazone is troglitazone, which was withdrawn from the market by the United States Food and Drug Administration (FDA) because of concerns about its safety. Muriglitazar, which stimulates both PPARγ and alpha receptors, increased adverse cardiovascular events and was also withdrawn by its manufacturer after rejection by the FDA. Roziglitazone and pioglitazone are also drugs in the PPARγ agonist family. Nissen *et al*[71] reported in a meta-analysis a significant increase in the risk of myocardial infarction with rosiglitazone and a trend towards increased risk of death from cardiovascular causes. This information has been includ-

Table 1 Effects of hypoglycemic drugs on ischemic preconditioning

Study	Model	Diabetic drug	Effect
Animal studies			
Gross *et al*[23], 1992	Dogs	Glibenclamide (glyburide)	Abolished
Toombs *et al*[50], 1993	Rabbits	Glibenclamide	Abolished
Mocanu *et al*[47], 2001	Rats	Glimepiride	Preserved
Maddock *et al*[51], 2004	Rats	Glibenclamide	Abolished
		Glimepiride	Preserved
Hausenloy *et al*[61], 2013	Rats	Glimepiride	Preserved
Ye *et al*[62], 2008	Rats	Pioglitazone	Preserved
		Glibenclamide (glyburide)	Abolished
		Glimepiride	Preserved
Horimoto *et al*[63], 2002	Rabbits	Glibenclamide	Abolished
		Glimepiride	Preserved
Bose *et al*[69], 2005	Rats	Native sequenced human GLP-1	Preserved
Zhu *et al*[73], 2011	Rats	Pioglitazone	IPC mimic
Sasaki *et al*[74], 2007	Rats	Pioglitazone	IPC mimic
Ahmed *et al*[75], 2011	Rats	Pioglitazone	IPC mimic
Li *et al*[76], 2008	Rats	Pioglitazone	Preserved
Wynne *et al*[77], 2005	Rats	Pioglitazone	IPC mimic
Sarraf *et al*[78], 2012	Porcine	Pioglitazone	Abolished
		Rosiglitazone	Abolished
Human studies			
Cleveland *et al*[52], 1997	Atrial muscle trabeculae	Glibenclamide (glyburide)	Abolished
Tomai *et al*[53], 1994	Human	Glibenclamide	Abolished
Klepzig *et al*[45], 1999	Human	Glibenclamide	Abolished
		Glimepiride	Preserved
Lee *et al*[54], 2002	Human	Glibenclamide	Abolished
Tomai *et al*[55], 1999	Human	Glibenclamide	Abolished
Ovünç[56], 2000	Human	Glibenclamide	Abolished
Ferreira *et al*[57], 2005	Human	Glibenclamide	Abolished
Bilinska *et al*[58], 2007	Human	Glibenclamide	Abolished
		Gliclazide	Partially preserved
Bogaty *et al*[59], 1998	Human	Glibenclamide	Preserved
Correa *et al*[60], 1997	Human	Glibenclamide	Preserved
Loubani *et al*[64], 2005	Right atrial appendages	Glibenclamide	Abolished
		Gliclazide	Preserved (but abolished in supratherapeutic concentrations)
Hueb *et al*[66], 2007	Human	Repaglinide	Abolished
Rahmi *et al*[70], 2013	Human	Repaglinide	Abolished
		Vildagliptin	Preserved

GLP-1: Glucagon-like peptide-1; IPC: Ischemic preconditioning.

ed in the prescribing information for all rosiglitazone-containing products. However, the glitazones have been shown to improve many of the traditional as well as the emerging risk factors associated with CVD[72]. The effect of the glitazones, rosiglitazone, and pioglitazone on IPC is still a matter of debate in the literature, as experimental studies demonstrate contradictory results. Methodological differences are one of the reasons for that. In studies using rat models, pioglitazone was associated with beneficial effects on cardiomyocyte injury, limiting infarct size, and ventricular arrhythmias[73-75]. These beneficial effects may be related to the opening of mitochondrial (ATP)-sensitive potassium channels[76] and by other kinases like phosphatidylinositol 3 kinase and P42/44 MAPK by pioglitazone[77]. On the other hand, in a porcine model, pioglitazone and rosiglitazone had the opposite results[78]. Finally, in the clinical setting, the possible actions of the glitazones on IPC are still uncertain.

EFFECTS OF METFORMIN ON IPC

The cardiovascular benefits observed in diabetic patients with chronic coronary artery disease with the use of metformin[79] have also been observed in experimental studies, which have shown positive results of metformin in the cardiovascular system, and that includes its effect in IPC. It is still not completely understood how metformin protects IPC in the heart, but it is postulated that it activates some kinases involved in IPC, such as (AMP)-activated protein kinase[80], which increases adenosine, activating cardioprotective mechanisms. Recent studies have also demonstrated that metformin increases hexokinase Ⅱ, another important kinase found in mitochondria, which seems to be one of the end-effectors of IPC, and that ultimately protects many cell types, including cardiomyocytes, against apoptosis and ischemic cell death[81]. Ischemia inhibits the loss of hexokinase Ⅱ from mitochondria, consequently preventing the opening of the mitochondrial permeability transition pore. This pore is responsible for the stabilization of the mitochondrial membrane potential, the prevention of cytochrome C release and also the reduction in reactive oxygen species production, which all finally lead to mitochondrial protection against ischemic injury[82,83]. These actions

associated with metabolic alterations, such as the prevention of acidosis through enhanced coupling of glycolysis and glucose oxidation and inhibition of fatty acid oxidation[81], are the responsible pathways by which metformin protects the myocardium from ischemia, in addition to its well-known effects in glucose control.

CLINICAL IMPLICATIONS

Ischemic preconditioning is a complex, dynamic phenomenon that can be the target of drug activities affecting the heart's ability to adapt to ischemic stress. In the clinical setting, however, the literature contains conflicting results regarding whether the use of conventional oral hypoglycemic agents affect cardiovascular mortality[84-90]. The findings from studies about the effects of hypoglycemic drugs on IPC have implications for diabetic patients, especially for those with a high risk of myocardial ischemic events, because the results infer that the myocardium may or may not benefit from a cardioprotective response when under the influence of such drugs. The most important consideration in this matter is that therapeutic options for diabetes treatment go beyond glucose-lowering efficacy in populations with increased risk of coronary ischemic events, and further large clinical trials will be necessary to determine whether the interference with myocardial preconditioning translates into clinical evidence.

REFERENCES

1 **Danaei G**, Finucane MM, Lu Y, Singh GM, Cowan MJ, Paciorek CJ, Lin JK, Farzadfar F, Khang YH, Stevens GA, Rao M, Ali MK, Riley LM, Robinson CA, Ezzati M. National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2·7 million participants. *Lancet* 2011; **378**: 31-40 [PMID: 21705069 DOI: 10.1016/S0140-6736(11)60679-X]

2 **Roglic G**, Unwin N. Mortality attributable to diabetes: estimates for the year 2010. *Diabetes Res Clin Pract* 2010; **87**: 15-19 [PMID: 19914728 DOI: 10.1016/j.diabres.2009]

3 **Kirtane AJ**, Ellis SG, Dawkins KD, Colombo A, Grube E, Popma JJ, Fahy M, Leon MB, Moses JW, Mehran R, Stone GW. Paclitaxel-eluting coronary stents in patients with diabetes mellitus: pooled analysis from 5 randomized trials. *J Am Coll Cardiol* 2008; **51**: 708-715 [PMID: 18279734 DOI: 10.1016/j.jacc2007.10.035]

4 **Cubbon RM**, Wheatcroft SB, Grant PJ, Gale CP, Barth JH, Sapsford RJ, Ajjan R, Kearney MT, Hall AS. Temporal trends in mortality of patients with diabetes mellitus suffering acute myocardial infarction: a comparison of over 3000 patients between 1995 and 2003. *Eur Heart J* 2007; **28**: 540-545 [PMID: 17289742 DOI: 10.1093/eurheartj/ehj510]

5 **Norhammar A**, Lindbäck J, Rydén L, Wallentin L, Stenestrand U. Improved but still high short- and long-term mortality rates after myocardial infarction in patients with diabetes mellitus: a time-trend report from the Swedish Register of Information and Knowledge about Swedish Heart Intensive Care Admission. *Heart* 2007; **93**: 1577-1583 [PMID: 17237125 DOI: 10.1136/hrt.2006.097956]

6 **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]

7 The University Group Diabetes Program. A study of the effects of hypoglycemic agents on vascular complications in patients with adult-onset diabetes. V. Evaluation of phenformin therapy. *Diabetes* 1975; **24** Suppl 1: 65-184 [PMID: 1090475]

8 **Murry CE**, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 1986; **74**: 1124-1136 [PMID: 3769170 DOI: 10.1161/01.CIR.74.5.1124]

9 **Baxter GF**, Goma FM, Yellon DM. Characterisation of the infarct-limiting effect of delayed preconditioning: timecourse and dose-dependency studies in rabbit myocardium. *Basic Res Cardiol* 1997; **92**: 159-167 [PMID: 9226101 DOI: 10.1007/BF00788633]

10 **Shiki K**, Hearse DJ. Preconditioning of ischemic myocardium: reperfusion-induced arrhythmias. *Am J Physiol* 1987; **253**: H1470-H1476 [PMID: 3425747]

11 **Cohen MV**, Liu GS, Downey JM. Preconditioning causes improved wall motion as well as smaller infarcts after transient coronary occlusion in rabbits. *Circulation* 1991; **84**: 341-349 [PMID: 2060104 DOI: 10.1161/01.CIR.84.1.34]

12 **Ikonomidis JS**, Tumiati LC, Weisel RD, Mickle DA, Li RK. Preconditioning human ventricular cardiomyocytes with brief periods of simulated ischaemia. *Cardiovasc Res* 1994; **28**: 1285-1291 [PMID: 7954635 DOI: 10.1093/cvr/28.8.1285]

13 **Hassouna A**, Loubani M, Matata BM, Fowler A, Standen NB, Galiñanes M. Mitochondrial dysfunction as the cause of the failure to precondition the diabetic human myocardium. *Cardiovasc Res* 2006; **69**: 450-458 [PMID: 16330008 DOI: 10.1016/j.cardiores.2005.11.004]

14 **Downey JM**, Davis AM, Cohen MV. Signaling pathways in ischemic preconditioning. *Heart Fail Rev* 2007; **12**: 181-188 [PMID: 17516169 DOI: 10.1007/s10741-007-9025-2]

15 **Hausenloy DJ**, Yellon DM. Preconditioning and postconditioning: united at reperfusion. *Pharmacol Ther* 2007; **116**: 173-191 [PMID: 17681609 DOI: 10.1016/j.pharmthera.2007.06.005]

16 **Liu GS**, Thornton J, Van Winkle DM, Stanley AW, Olsson RA, Downey JM. Protection against infarction afforded by preconditioning is mediated by A1 adenosine receptors in rabbit heart. *Circulation* 1991; **84**: 350-356 [PMID: 2060105 DOI: 10.1161/01.CIR.84.1.350]

17 **Wall TM**, Sheehy R, Hartman JC. Role of bradykinin in myocardial preconditioning. *J Pharmacol Exp Ther* 1994; **270**: 681-689 [PMID: 8071859]

18 **Schultz JE**, Rose E, Yao Z, Gross GJ. Evidence for involvement of opioid receptors in ischemic preconditioning in rat hearts. *Am J Physiol* 1995; **268**: H2157-H2161 [PMID: 7771566]

19 **Wan TC**, Ge ZD, Tampo A, Mio Y, Bienengraeber MW, Tracey WR, Gross GJ, Kwok WM, Auchampach JA. The A3 adenosine receptor agonist CP-532,903 [N6-(2,5-dichlorobenzyl)-3′-aminoadenosine-5′-N-methylcarboxamide] protects against myocardial ischemia/reperfusion injury via the sarcolemmal ATP-sensitive potassium channel. *J Pharmacol Exp Ther* 2008; **324**: 234-243 [PMID: 17906066 DOI: 10.1124/jpet.107.127480]

20 **Quinlan CL**, Costa AD, Costa CL, Pierre SV, Dos Santos P, Garlid KD. Conditioning the heart induces formation of signalosomes that interact with mitochondria to open mitoKATP channels. *Am J Physiol Heart Circ Physiol* 2008; **295**: H953-H961 [PMID: 18621853 DOI: 10.1152/ajpheart.00520.2008]

21 **Ytrehus K**, Liu Y, Downey JM. Preconditioning protects ischemic rabbit heart by protein kinase C activation. *Am J Physiol* 1994; **266**: H1145-H1152 [PMID: 8160817]

22 **Marinovic J**, Ljubkovic M, Stadnicka A, Bosnjak ZJ, Bienengraeber M. Role of sarcolemmal ATP-sensitive potassium channel in oxidative stress-induced apoptosis:

mitochondrial connection. *Am J Physiol Heart Circ Physiol* 2008; **294**: H1317-H1325 [PMID: 18192220 DOI: 10.1152/ajpheart.00840.2007]
23 **Gross GJ**, Auchampach JA. Blockade of ATP-sensitive potassium channels prevents myocardial preconditioning in dogs. *Circ Res* 1992; **70**: 223-233 [PMID: 1310443 DOI: 10.1161/01.RES.70.2.223]
24 **Grover GJ**, Sleph PG, Dzwonczyk S. Role of myocardial ATP-sensitive potassium channels in mediating preconditioning in the dog heart and their possible interaction with adenosine A1-receptors. *Circulation* 1992; **86**: 1310-1316 [PMID: 1394937 DOI: 10.1161/01.CIR.86.4.1310]
25 **Schulz R**, Rose J, Heusch G. Involvement of activation of ATP-dependent potassium channels in ischemic preconditioning in swine. *Am J Physiol* 1994; **267**: H1341-H1352 [PMID: 7943380]
26 **Van Winkle DM**, Chien GL, Wolff RA, Soifer BE, Kuzume K, Davis RF. Cardioprotection provided by adenosine receptor activation is abolished by blockade of the KATP channel. *Am J Physiol* 1994; **266**: H829-H839 [PMID: 8141383]
27 **Penson PE**, Ford WR, Kidd EJ, Broadley KJ. Activation of beta-adrenoceptors mimics preconditioning of rat-isolated atria and ventricles against ischaemic contractile dysfunction. *Naunyn Schmiedebergs Arch Pharmacol* 2008; **378**: 589-597 [PMID: 18663429 DOI: 10.1007/s00210-008-0331-6]
28 **Suematsu Y**, Anttila V, Takamoto S, del Nido P. Cardioprotection afforded by ischemic preconditioning interferes with chronic beta-blocker treatment. *Scand Cardiovasc J* 2004; **38**: 293-299 [PMID: 15513313 DOI: 10.1080/14017430410021507]
29 **Cain BS**, Meldrum DR, Cleveland JC, Meng X, Banerjee A, Harken AH. Clinical L-type Ca(2+) channel blockade prevents ischemic preconditioning of human myocardium. *J Mol Cell Cardiol* 1999; **31**: 2191-2197 [PMID: 10640446 DOI: 10.1006/jmcc.1999.1039]
30 **Gross ER**, Hsu AK, Gross GJ. Acute aspirin treatment abolishes, whereas acute ibuprofen treatment enhances morphine-induced cardioprotection: role of 12-lipoxygenase. *J Pharmacol Exp Ther* 2004; **310**: 185-191 [PMID: 14993258 DOI: 10.1124/jpet.103.064667]
31 **Jancso G**, Cserepes B, Gasz B, Benko L, Ferencz A, Borsiczky B, Lantos J, Dureja A, Kiss K, Szeberényi J, Roth E. Effect of acetylsalicylic acid on nuclear factor-kappaB activation and on late preconditioning against infarction in the myocardium. *J Cardiovasc Pharmacol* 2005; **46**: 295-301 [PMID: 16116334]
32 **Hiraoka M**, Fan Z. Activation of ATP-sensitive outward K+ current by nicorandil (2-nicotinamidoethyl nitrate) in isolated ventricular myocytes. *J Pharmacol Exp Ther* 1989; **250**: 278-285 [PMID: 2526215]
33 **IONA Study Group**. Effect of nicorandil on coronary events in patients with stable angina: the Impact Of Nicorandil in Angina (IONA) randomised trial. *Lancet* 2002; **359**: 1269-1275 [PMID: 11965271 DOI: 10.1016/S0140-6736(02)08265-X]
34 **Schmid-Antomarchi H**, De Weille J, Fosset M, Lazdunski M. The receptor for antidiabetic sulfonylureas controls the activity of the ATP-modulated K+ channel in insulin-secreting cells. *J Biol Chem* 1987; **262**: 15840-15844 [PMID: 2445740]
35 **Hasselblatt A**. Sulfonylureas: pharmacokinetics in animal experiments. In: Kuhlmann J, Puls W. Handbook of Experimental Pharmacology. Oral Antidiabetics. Berlin/Heidelberg, Springer Verlag, 1996: 161-183
36 **Noma A**. ATP-regulated K+ channels in cardiac muscle. *Nature* 1983; **305**: 147-148 [PMID: 6310409 DOI: 10.1038/305147a0]
37 **Inagaki N**, Gonoi T, Clement JP, Wang CZ, Aguilar-Bryan L, Bryan J, Seino S. A family of sulfonylurea receptors determines the pharmacological properties of ATP-sensitive K+ channels. *Neuron* 1996; **16**: 1011-1017 [PMID: 8630239 DOI: 10.1016/S0896-6273(00)80124-5]
38 **Standen NB**, Quayle JM, Davies NW, Brayden JE, Huang Y, Nelson MT. Hyperpolarizing vasodilators activate ATP-sensitive K+ channels in arterial smooth muscle. *Science* 1989; **245**: 177-180 [PMID: 2501869 DOI: 10.1126/science.2501869]
39 **Nichols CG**, Lederer WJ. Adenosine triphosphate-sensitive potassium channels in the cardiovascular system. *Am J Physiol* 1991; **261**: H1675-H1686 [PMID: 1750525]
40 **Gribble FM**, Reimann F. Sulphonylurea action revisited: the post-cloning era. *Diabetologia* 2003; **46**: 875-891 [PMID: 12819907 DOI: 10.1007/s00125-003-1143-3]
41 **Ashfield R**, Gribble FM, Ashcroft SJ, Ashcroft FM. Identification of the high-affinity tolbutamide site on the SUR1 subunit of the K(ATP) channel. *Diabetes* 1999; **48**: 1341-1347 [PMID: 10342826 DOI: 10.2337/diabetes.48.6.1341]
42 **Gribble FM**, Ashcroft FM. Differential sensitivity of beta-cell and extrapancreatic K(ATP) channels to gliclazide. *Diabetologia* 1999; **42**: 845-848 [PMID: 10440127 DOI: 10.1007/s001250051236]
43 **Abdelmoneim AS**, Hasenbank SE, Seubert JM, Brocks DR, Light PE, Simpson SH. Variations in tissue selectivity amongst insulin secretagogues: a systematic review. *Diabetes Obes Metab* 2012; **14**: 130-138 [PMID: 21923736 DOI: 10.1111/j.1463-1326.2011.01496.x]
44 **Song DK**, Ashcroft FM. Glimepiride block of cloned beta-cell, cardiac and smooth muscle K(ATP) channels. *Br J Pharmacol* 2001; **133**: 193-199 [PMID: 11325810 DOI: 10.1038/sj.bjp.0704062]
45 **Klepzig H**, Kober G, Matter C, Luus H, Schneider H, Boedeker KH, Kiowski W, Amann FW, Gruber D, Harris S, Burger W. Sulfonylureas and ischaemic preconditioning; a double-blind, placebo-controlled evaluation of glimepiride and glibenclamide. *Eur Heart J* 1999; **20**: 439-446 [PMID: 10213347 DOI: 10.1053/euhj.1998.1242]
46 **Lee TM**, Chou TF. Impairment of myocardial protection in type 2 diabetic patients. *J Clin Endocrinol Metab* 2003; **88**: 531-537 [PMID: 12574175 DOI: 10.1210/jc.2002-020904]
47 **Mocanu MM**, Maddock HL, Baxter GF, Lawrence CL, Standen NB, Yellon DM. Glimepiride, a novel sulfonylurea, does not abolish myocardial protection afforded by either ischemic preconditioning or diazoxide. *Circulation* 2001; **103**: 3111-3116 [PMID: 11425777 DOI: 10.1161/01.CIR.103.25.3111]
48 **Wu GT**, Wang L, Li J, Zhu WZ. Effects of glibenclamide, glimepiride, and gliclazide on ischemic preconditioning in rat heart. *Chin Med Sci J* 2007; **22**: 162-168 [PMID: 17966164]
49 **Gribble FM**, Tucker SJ, Seino S, Ashcroft FM. Tissue specificity of sulfonylureas: studies on cloned cardiac and beta-cell K(ATP) channels. *Diabetes* 1998; **47**: 1412-1418 [PMID: 9726229 DOI: 10.2337/diabetes.47.9.1412]
50 **Toombs CF**, Moore TL, Shebuski RJ. Limitation of infarct size in the rabbit by ischaemic preconditioning is reversible with glibenclamide. *Cardiovasc Res* 1993; **27**: 617-622 [PMID: 8324795 DOI: 10.1093/cvr/27.4.617]
51 **Maddock HL**, Siedlecka SM, Yellon DM. Myocardial protection from either ischaemic preconditioning or nicorandil is not blocked by gliclazide. *Cardiovasc Drugs Ther* 2004; **18**: 113-119 [PMID: 15162072 DOI: 10.1023/B:CARD.0000029028.75316.5e]
52 **Cleveland JC**, Meldrum DR, Cain BS, Banerjee A, Harken AH. Oral sulfonylurea hypoglycemic agents prevent ischemic preconditioning in human myocardium. Two paradoxes revisited. *Circulation* 1997; **96**: 29-32 [PMID: 9236412 DOI: 10.1161/01.CIR.96.1.29]
53 **Tomai F**, Crea F, Gaspardone A, Versaci F, De Paulis R, Penta de Peppo A, Chiariello L, Gioffrè PA. Ischemic preconditioning during coronary angioplasty is prevented by glibenclamide, a selective ATP-sensitive K+ channel blocker. *Circulation* 1994; **90**: 700-705 [PMID: 8044938 DOI: 10.1161/01.CIR.90.2.700]
54 **Lee TM**, Su SF, Chou TF, Lee YT, Tsai CH. Loss of preconditioning by attenuated activation of myocardial ATP-sensitive potassium channels in elderly patients undergoing coronary angioplasty. *Circulation* 2002; **105**: 334-340 [PMID: 11804989

DOI: 10.1161/hc0302.102572]
55 **Tomai F**, Danesi A, Ghini AS, Crea F, Perino M, Gaspardone A, Ruggeri G, Chiariello L, Gioffrè PA. Effects of K(ATP) channel blockade by glibenclamide on the warm-up phenomenon. *Eur Heart J* 1999; **20**: 196-202 [PMID: 10082152 DOI: 10.1053/euhj.1998.1311]
56 **Ovünç K**. Effects of glibenclamide, a K(ATP) channel blocker, on warm-up phenomenon in type II diabetic patients with chronic stable angina pectoris. *Clin Cardiol* 2000; **23**: 535-539 [PMID: 10894443]
57 **Ferreira BM**, Moffa PJ, Falcão A, Uchida A, Camargo P, Pereyra P, Soares PR, Hueb W, Ramires JA. The effects of glibenclamide, a K(ATP) channel blocker, on the warm-up phenomenon. *Ann Noninvasive Electrocardiol* 2005; **10**: 356-362 [PMID: 16029388 DOI: 10.1111/j.1542-474X.2005.00650.x]
58 **Bilinska M**, Potocka J, Korzeniowska-Kubacka I, Piotrowicz R. 'Warm-up' phenomenon in diabetic patients with stable angina treated with diet or sulfonylureas. *Coron Artery Dis* 2007; **18**: 455-462 [PMID: 17700217 DOI: 10.1097/MCA.0b013e3282a30676]
59 **Bogaty P**, Kingma JG, Robitaille NM, Plante S, Simard S, Charbonneau L, Dumesnil JG. Attenuation of myocardial ischemia with repeated exercise in subjects with chronic stable angina: relation to myocardial contractility, intensity of exercise and the adenosine triphosphate-sensitive potassium channel. *J Am Coll Cardiol* 1998; **32**: 1665-1671 [PMID: 9822094 DOI: 10.1016/S0735-1097(98)00431-8]
60 **Correa SD**, Schaefer S. Blockade of K(ATP) channels with glibenclamide does not abolish preconditioning during demand ischemia. *Am J Cardiol* 1997; **79**: 75-78 [PMID: 9024742 DOI: 10.1016/S0002-9149(96)00681-9]
61 **Hausenloy DJ**, Wynne AM, Mocanu MM, Yellon DM. Glimepiride treatment facilitates ischemic preconditioning in the diabetic heart. *J Cardiovasc Pharmacol Ther* 2013; **18**: 263-269 [PMID: 23263382 DOI: 10.1177/1074248412468945]
62 **Ye Y**, Lin Y, Perez-Polo JR, Birnbaum Y. Oral glyburide, but not glimepiride, blocks the infarct-size limiting effects of pioglitazone. *Cardiovasc Drugs Ther* 2008; **22**: 429-436 [PMID: 18825491 DOI: 10.1007/s10557-008-6138-3]
63 **Horimoto H**, Nakai Y, Mieno S, Nomura Y, Nakahara K, Sasaki S. Oral hypoglycemic sulfonylurea glimepiride preserves the myoprotective effects of ischemic preconditioning. *J Surg Res* 2002; **105**: 181-188 [PMID: 12121705 DOI: 10.1006/jsre.2002.6379]
64 **Loubani M**, Fowler A, Standen NB, Galiñanes M. The effect of gliclazide and glibenclamide on preconditioning of the human myocardium. *Eur J Pharmacol* 2005; **515**: 142-149 [PMID: 15894305 DOI: 10.1016/j.ejphar.2005.04.002]
65 **Dabrowski M**, Wahl P, Holmes WE, Ashcroft FM. Effect of repaglinide on cloned beta cell, cardiac and smooth muscle types of ATP-sensitive potassium channels. *Diabetologia* 2001; **44**: 747-756 [PMID: 11440368 DOI: 10.1007/s001250051684]
66 **Hueb W**, Uchida AH, Gersh BJ, Betti RT, Lopes N, Moffa PJ, Ferreira BM, Ramires JA, Wajchenberg BL. Effect of a hypoglycemic agent on ischemic preconditioning in patients with type 2 diabetes and stable angina pectoris. *Coron Artery Dis* 2007; **18**: 55-59 [PMID: 17172931 DOI: 10.1097/MCA.0b013e328011c0a9]
67 **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]
68 **Ahrén B**. Dipeptidyl peptidase-4 inhibitors: clinical data and clinical implications. *Diabetes Care* 2007; **30**: 1344-1350 [PMID: 17337494 DOI: 10.2337/dc07-0233]
69 **Bose AK**, Mocanu MM, Carr RD, Yellon DM. Glucagon like peptide-1 is protective against myocardial ischemia/reperfusion injury when given either as a preconditioning mimetic or at reperfusion in an isolated rat heart model. *Cardiovasc Drugs Ther* 2005; **19**: 9-11 [PMID: 15883751 DOI: 10.1007/s10557-005-6892-4]
70 **Rahmi RM**, Uchida AH, Rezende PC, Lima EG, Garzillo CL, Favarato D, Strunz CM, Takiuti M, Girardi P, Hueb W, Kalil Filho R, Ramires JA. Effect of hypoglycemic agents on ischemic preconditioning in patients with type 2 diabetes and symptomatic coronary artery disease. *Diabetes Care* 2013; **36**: 1654-1659 [PMID: 23250803 DOI: 10.2337/dc12-1495]
71 **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]
72 **Parulkar AA**, Pendergrass ML, Granda-Ayala R, Lee TR, Fonseca VA. Nonhypoglycemic effects of thiazolidinediones. *Ann Intern Med* 2001; **134**: 61-71 [PMID: 11187421]
73 **Zhu QW**, Wang H, Zhang JY, Ye P, Luo LM. [Effect of pioglitazone on hypoxia/reoxygenation injury and protein kinase C expression in neonatal rat cardiomyocytes]. *Nanfang Yike Daxue Xuebao* 2011; **31**: 1819-1823 [PMID: 22126757]
74 **Sasaki H**, Ogawa K, Shimizu M, Mori C, Takatsuka H, Okazaki F, Kawai M, Taniguchi I, Mochizuki S. The insulin sensitizer pioglitazone improves the deterioration of ischemic preconditioning in type 2 diabetes mellitus rats. *Int Heart J* 2007; **48**: 623-635 [PMID: 17998772 DOI: 10.1536/ihj.48.623]
75 **Ahmed LA**, Salem HA, Attia AS, Agha AM. Pharmacological preconditioning with nicorandil and pioglitazone attenuates myocardial ischemia/reperfusion injury in rats. *Eur J Pharmacol* 2011; **663**: 51-58 [PMID: 21549700 DOI: 10.1016/j.ejphar.2011.04.038]
76 **Li J**, Lang MJ, Mao XB, Tian L, Feng YB. Antiapoptosis and mitochondrial effect of pioglitazone preconditioning in the ischemic/reperfused heart of rat. *Cardiovasc Drugs Ther* 2008; **22**: 283-291 [PMID: 18470603 DOI: 10.1007/s10557-008-6115-x]
77 **Wynne AM**, Mocanu MM, Yellon DM. Pioglitazone mimics preconditioning in the isolated perfused rat heart: a role for the prosurvival kinases PI3K and P42/44MAPK. *J Cardiovasc Pharmacol* 2005; **46**: 817-822 [PMID: 16306807]
78 **Sarraf M**, Lu L, Ye S, Reiter MJ, Greyson CR, Schwartz GG. Thiazolidinedione drugs promote onset, alter characteristics, and increase mortality of ischemic ventricular fibrillation in pigs. *Cardiovasc Drugs Ther* 2012; **26**: 195-204 [PMID: 22450779 DOI: 10.1007/s10557-012-6384-2]
79 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 DOI: 10.1016/S0140-6736(98)07037-8]
80 **Solskov L**, Magnusson NE, Kristiansen SB, Jessen N, Nielsen TT, Schmitz O, Bøtker HE, Lund S. Microarray expression analysis in delayed cardioprotection: the effect of exercise, AICAR, or metformin and the possible role of AMP-activated protein kinase (AMPK). *Mol Cell Biochem* 2012; **360**: 353-362 [PMID: 21964537 DOI: 10.1007/s11010-011-1075-z]
81 **Nederlof R**, Eerbeek O, Hollmann MW, Southworth R, Zuurbier CJ. Targeting hexokinase II to mitochondria to modulate energy metabolism and reduce ischaemia-reperfusion injury in heart. *Br J Pharmacol* 2014; **171**: 2067-2079 [PMID: 24032601 DOI: 10.1111/bph.12363];]
82 **Pasdois P**, Parker JE, Halestrap AP. Extent of mitochondrial hexokinase II dissociation during ischemia correlates with mitochondrial cytochrome c release, reactive oxygen species production, and infarct size on reperfusion. *J Am Heart Assoc* 2013; **2**: e005645 [PMID: 23525412 DOI: 10.1161/JAHA.112.005645]
83 **El Messaoudi S**, Rongen GA, de Boer RA, Riksen NP. The cardioprotective effects of metformin. *Curr Opin Lipidol* 2011; **22**: 445-453 [PMID: 21897229 DOI: 10.1097/MOL.0b013e32834ae1a7]
84 **Garratt KN**, Brady PA, Hassinger NL, Grill DE, Terzic A, Holmes DR. Sulfonylurea drugs increase early mortality in patients with diabetes mellitus after direct angioplasty for acute myocardial infarction. *J Am Coll Cardiol* 1999; **33**:

119-124 [PMID: 9935017 DOI: 10.1016/S0735-1097(98)00557-9]
85 **Johnson JA**, Majumdar SR, Simpson SH, Toth EL. Decreased mortality associated with the use of metformin compared with sulfonylurea monotherapy in type 2 diabetes. *Diabetes Care* 2002; **25**: 2244-2248 [PMID: 12453968 DOI: 10.2337/diacare.25.12.2244]
86 **Danchin N**, Charpentier G, Ledru F, Vaur L, Guéret P, Hanania G, Blanchard D, Lablanche JM, Genès N, Cambou JP. Role of previous treatment with sulfonylureas in diabetic patients with acute myocardial infarction: results from a nationwide French registry. *Diabetes Metab Res Rev* 2005; **21**: 143-149 [PMID: 15386810 DOI: 10.1002/dmrr.498]
87 **Meier JJ**, Gallwitz B, Schmidt WE, Mügge A, Nauck MA. Is impairment of ischaemic preconditioning by sulfonylurea drugs clinically important? *Heart* 2004; **90**: 9-12 [PMID: 14676228 DOI: 10.1136/heart.90.1.9]
88 **Jørgensen CH**, Gislason GH, Andersson C, Ahlehoff O, Charlot 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]
89 **Pantalone KM**, Kattan MW, Yu C, Wells BJ, Arrigain S, Jain A, Atreja A, Zimmerman RS. Increase in overall mortality risk in patients with type 2 diabetes receiving glipizide, glyburide or glimepiride monotherapy versus metformin: a retrospective analysis. *Diabetes Obes Metab* 2012; **14**: 803-809 [PMID: 22486923 DOI: 10.1111/j.1463-1326.2012.01604.x]
90 **Schramm TK**, Gislason GH, Vaag A, Rasmussen JN, Folke F, Hansen ML, Fosbøl EL, Køber L, Norgaard ML, Madsen M, Hansen PR, Torp-Pedersen C. Mortality and cardiovascular risk associated with different insulin secretagogues compared with metformin in type 2 diabetes, with or without a previous myocardial infarction: a nationwide study. *Eur Heart J* 2011; **32**: 1900-1908 [PMID: 21471135 DOI: 10.1093/eurheartj/ehr077]

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TOPIC HIGHLIGHT

WJD 5th Anniversary Special Issues (2): Type 2 diabetes

Functional foods-based diet as a novel dietary approach for management of type 2 diabetes and its complications: A review

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Abstract

Type 2 diabetes is a complicated metabolic disorder with both short- and long-term undesirable complications. In recent years, there has been growing evidence that functional foods and their bioactive compounds, due to their biological properties, may be used as complementary treatment for type 2 diabetes mellitus. In this review, we have highlighted various functional foods as missing part of medical nutrition therapy in diabetic patients. Several *in vitro*, animal models and some human studies, have demonstrated that functional foods and nutraceuticals may improve postprandial hyperglycemia and adipose tissue metabolism modulate carbohydrate and lipid metabolism. Functional foods may also improve dyslipidemia and insulin resistance, and attenuate oxidative stress and inflammatory processes and subsequently could prevent the development of long-term diabetes complications including cardiovascular disease, neuropathy, nephropathy and retinopathy. In conclusion available data indicate that a functional foods-based diet may be a novel and comprehensive dietary approach for management of type 2 diabetes.

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Key words: Type 2 diabetes; Insulin resistance; Functional foods; Whole grain; Legumes; Nuts; Fruits; Herbs or spices; Vegetables; Prebiotics; Probiotics

Core tip: Medical nutrition therapy (MNT) is a main part of type 2 diabetes management. Apparently the therapeutic and medicinal properties of foods maybe a missing step during MNT process, and could enhance the effectiveness of dietary management of type 2 diabetes.

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INTRODUCTION

Type 2 diabetes is a metabolic disorder characterized by hyperglycemia, developing insulin resistance, β-cell dysfunction and impaired insulin secretion[1,2]. Multiple metabolic disorders including impaired lipid and lipoprotein metabolism, oxidative stress (over production of free

radicals and defect in endogenous antioxidant defense system), sub-clinical inflammation, vascular endothelial dysfunction and hypertension are commonly accompanied by type 2 diabetes[3-5]; these metabolic disorders lead to long-term pathogenic conditions such as micro- and macro-vascular complications including neuropathy, retinopathy, nephropathy, and a decreased quality of life and an increased mortality rate[6,7].

Despite availability of many pharmacological interventions including oral hypoglycemic agents and insulin therapy for diabetes management, current evidence shows an alarming rising trend in the occurrence of undesirable complications among these patients[1].

Medical nutrition therapy (MNT) is also a main part of type 2 diabetes management; estimation of energy and nutrients requirements, carbohydrate counting as well as glycemic index and glycemic load, recommendation for dietary fats and cholesterol and protein intakes, explanation the foods exchange list for patients and common important recommendations for a healthy diet are the main components of diet planning in type 2 diabetic patients[8,9]; however it is not clear whether this approach *per se* is sufficiently adequate for prevention of long-term complications of diabetes. Administration of various supplements, including antioxidant vitamins, fibers, ω_3 fatty acids, numerous nutraceuticals, and herbs has also been proposed for glycemic control but data available supporting these recommendations for diabetic patients are insufficient[10-14]. Apparently the therapeutic and medicinal properties of foods maybe a missing step during MNT process, and could enhance the effectiveness of dietary management of type 2 diabetes.

During the past two decades, the concept of functional food is fast expanding; functional foods beyond the basic nutritional functions have potential benefits to promote health and reduce the risk of chronic diseases and have hence been given much attention[15,16]. In recent years, researchers have focused on properties of the bioactive compounds of functional foods in the control of various aspects of diabetes mellitus; some protective effects of these compounds and food sources have been investigated *in vitro* and *in vivo*, and several clinical trials have even confirmed these advantages in diabetic patients[17-19].

Here, based on the multiple biological properties of functional foods and their bioactive compounds, a functional foods-based diet has been hypothesized as a novel and comprehensive dietary approach for management of type 2 diabetes and prevention of long-term complications.

RESEARCH

The evidence cited in this review was obtained through searches in PubMed, Scopus, and Google scholar using the following key words: "Type 2 diabetes or hyperglycemia", "insulin resistance", "cardiovascular disease", "obesity", "metabolic syndrome", "oxidative stress", "inflammation", long-term diabetic complications" in combination with "functional foods", "nutraceuticals", "bioactive food compounds", "fiber", "polyphenols", "whole grain", "legumes", "nuts", "fruits", "herbs or spices" "vegetables", "prebiotics", "probiotics", and "bioactive peptides". Relevant articles of acceptable quality were used. Briefly, in this article we tried to highlight some of the following important functional foods including whole grains, phytochemical-rich fruits and vegetables, legumes, nuts, dairy products, green tea and some spices, as required components of a health-promoting diet for diabetic patients.

Whole grains

Grains and cereal-based products are the basic sources providing energy and carbohydrate in human diets. Since the dietary carbohydrate sources in type 2 diabetic patients play a determining role in glycemic and insulin secretary response, the use of functional grains including whole grain cereals, and bakery products prepared using whole wheat, rye, oat, and barley is the first step in planning of a functional foods-based diet.

Some previous studies report that dietary carbohydrate modification in patients with metabolic syndrome resulted in favorable metabolic consequences especially increased insulin sensitivity, decreased adipocyte cell size, and modulated expression of adipose tissue genes involved in insulin signaling pathways (insulin-like-growth-factor binding protein-5, insulin receptors, hormone-sensitive lipase[20,21].

Compared to refined grains, whole grains (WGs) have more non-digestible complex polysaccharides including soluble and insoluble fibers, inulin, β-glucan, and resistant starches, as well as non-carbohydrate functional components including carotenoids, phytates and phytoesterogens, phenolic acids (ferulic acid, vanilic acid, caffeic acid, syringic acid, *P*-cumaric acid), and tocopherols. The most well-known protective effects of whole grain-based products against obesity, type 2 diabetes, cardiovascular diseases, hypertension, metabolic syndrome and various types of cancer, have been attributed to these bioactive compounds[22-25]. Among the several mechanisms available in current data regarding the beneficial effects of WGs and cereal-based products in diabetic patients, some of the more important are that bioactive compounds of WGs could effectively regulate glycemic response, increase insulin sensitivity, improve pancreatic β-cell functions and increase insulin secretion[26,27]. High contents of inulin and β-glucan, main soluble and fermentable fibers in WGs, in addition to their hypolipidemic and hypoglycemic effects, act as prebiotics in the gut and modulate gut microbiota *via* stimulation of growth and activity of bifidobacteria and lactic acid bacteria[28,29], effects leading to more metabolic responses (Figure 1).

Long-term follow-ups of diabetic patients indicate that higher consumption of whole grain, cereal fiber, bran, and germ were associated with decreased all-cause and cardiovascular disease-cause mortality[30]. Epidemiological studies also confirmed that regular consumption of WGs products could modify the main risk factors of atherosclerotic diseases including triglyceride and LDL-C

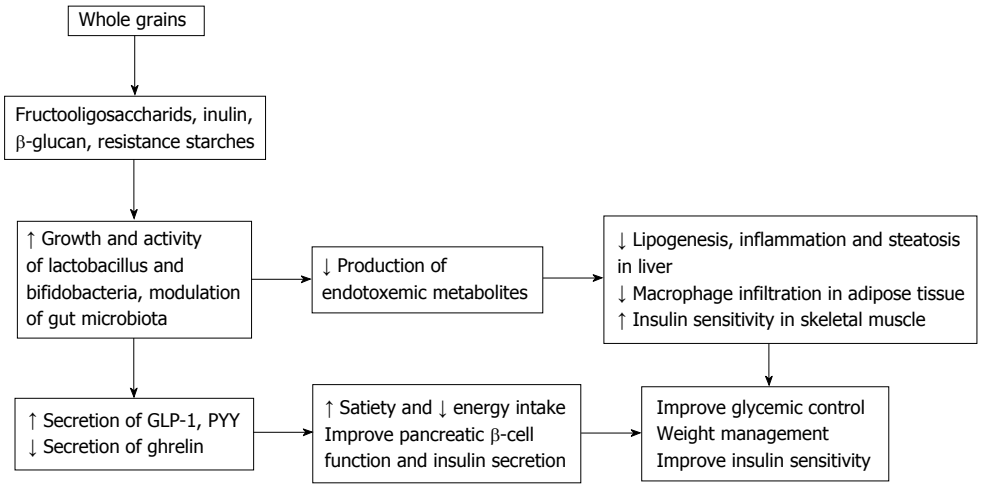

Figure 1 Role of prebiotic compounds of whole grains and cereal-based products in modulation of gut microbiota and con sequent metabolic effects could lead to better glycemic control.

levels, blood pressure and serum homocysteine levels, as well as vascular functions, and oxidative and inflammatory status[31].

Rye, a widely used grain especially in Northern and Eastern Europe, is considered a functional grain. The high fiber content of rye products decreases digestion and absorption of dietary carbohydrates, and increase metabolites derived from colonic fermentation of the soluble fiber of rye products, including propionic and butyric acids which effectively stimulate secretion of insulin from β-cells; studies have indicated that the bioactive compounds of rye (phenolic acids, tannins, benzoic acid, phenylalanine) derivates have a similar efficacy with antidiabetic drugs in insulin secretion[26,32]. In one study, the consumption of rye products in the breakfast meal increased colonic fermentation, decreased ghrelin levels and satiety rating in the late postprandial phase after breakfast as well as energy intake at a subsequent lunch meal, and improved acute glucose and insulin responses[32].

Oat meal products have also been investigated as healthy carbohydrate sources for diabetic patients; they are rich sources of soluble fiber especially β-glucan, antioxidants and bioactive compounds including carotenoids, phytic acid, phenolic acids (hydroxycinammic acids, caffeic acid, ferulic acid), flavonoids and phytosterols[33]. Studies show that consumption of oat products improves glycemic, insulinemic, and lipidemic responses in diabetic patients, and act as active ingredient reducing postprandial glycemia[34,35]. In diabetic animal models, oat products attenuated hyperglycemia-induced retinal oxidative stress, increased glycogen content of liver, decreased plasma free fatty acids and succinate dehydrogenase activity and inhibited pancreatic β-cell apoptosis as well[36].

The beneficial effects of barley and its by products for diabetic patients are mainly attributed to its high content of β-glucan; Administration of barley β-glucan extract in pre-diabetic subjects improved glucose tolerance and insulin resistance index[27]. In addition, barley may use as base of a meal; the use of barley combined with refined grains such as white rice maybe a practical way to attenuate their undesirable effects on glycemic control; in a randomized crossover study, combination of cooked barley with white rice dose-dependently reduced the area under the curves of plasma glucose and insulin concentrations, suppressed postprandial decrease of plasma desacyl ghrelin levels and consequently increased satiety[37]. The hypolipidemic properties, antioxidant and anti inflammatory activities of barley products have also been investigated[38,39]. In animal diabetic models, barley improved some features of fatty liver, decreased lipid content of the liver, increased fatty acid oxidation and adiponectin levels[40].

Several positive effects of whole wheat and its byproducts on carbohydrate and insulin metabolism have also been reported; wheat bran and whole wheat products are rich sources of dietary fiber, magnesium (main cofactor of enzymes involved in glucose metabolism and insulin secretion), potassium, phenolic acids, α-tocopherols, carotenoids and antioxidants[41]. It is believed that the majority of beneficial effects of whole wheat grain are related to bran and germ fractions; wheat bran is a main source of fiber, lignans, phenolic acid and alkylresorcinol, and beyond the health promotion of gastrointestinal tract and weight management, could improve postprandial glycemic response, glycosylated hemoglobin, lipid disorders and other cardiovascular risk factors in diabetic patients[42]. Studies showed that alkylresorcinol of wheat bran inhibited platelet activity and aggregation, decreased triglyceride de novo synthesis, and decreased cardiovascular disease risk factors[43]. Wheat germ is rich in non-digestible oligosaccharides, phytosterols, benzoquinone and flavonoids that play a potent role in induction of antioxidant and anti-inflammatory properties and modulation of immunity responses[44]. Avemar, fermented wheat germ extract, had interesting properties in the treatment of cardiovascular disease, and improved metabolic abnormalities including hyperglycemia, lipid peroxidation and abdominal fat gain[45].

Brown rice and its byproducts is another grain investigated as a functional food. Compared to white rice, brown rice has lower glycemic load and glycemic index, and higher content of fiber, vitamins and minerals, phytic acids, polyphenols, tocopherols, tocotrienols, and other bioactive compounds[46]; consumption of brown rice has benefits on glycemic control, dyslipidemia, endothelial function, abdominal obesity and liver functions in type 2 diabetic patients[47]. Studies show that γ-orizanol found in brown rice modulates high-fat diet induces oxidative stress, improves β-cell function, enhances glucose-stimulated insulin secretion and prevents the development of type 2 diabetes[48]. Germinated and pre-germinated brown rice, as more interesting functional foods, have unique components including γ-amino butyric acid, and bioactive acylated steryl glucosides with potent antidiabetic properties; these bioactive components attenuate oxidative-induced peripheral nervous system, prevent diabetic neuropathy, inhibit oxidative-induced pancreatic β-cell apoptosis and enhance insulin secretion[49-51]. Bran rice, a byproduct of brown rice, contains within 31% fiber (mainly insoluble fiber), β-glucan, pectin, tocopherols, orizanol, ferulic acid, lutein, xanthine, vitamin K, thiamin, niacin, pantothenic acid, α-lipoic acid, coenzyme Q_{10} and other nutraceuticals; administration of bran rice in diabetic patients reduced glycosylated hemoglobin, LDL-C and total cholesterol as well as increased HDL-C[52].

In conclusion, replacement of whole grain and cereal-based products with refined grains in diet planning may be an effective and practical strategy for MNT in type 2 diabetic patients; this approach beyond the improvement of glycemic control, leads to more benefits for management of other aspects of diabetes, attenuation of diabetes-induced metabolic disorders, and prevents long-term complications especially atherosclerosis and cardiovascular disease.

PHYTOCHEMICAL-RICH FRUITS AND VEGETABLES

Fruits and vegetables are rich sources of dietary fiber (soluble and insoluble fiber), vitamins, and various phytochemicals and play a vital role in health promotion and prevention of chronic disease[53]. Dietary modification based on fruits and vegetables certainly is a definitely important strategy for management of type 2 diabetes and prevention of its complications; several studies indicate that regular consumption of various fruits and vegetables in diabetic patients can lead to an improved glycemic control, reduced HbA1c and triglyceride levels, enhanced antioxidant defense system, attenuated oxidative stress and inflammatory markers, decreased risk of diabetic retinopathy, and a lower burden of carotid atherosclerosis[54-57]. Since various fruits and vegetables provide many different micronutrients and bioactive compounds, consumption of varied fruits and vegetables is mainly recommended; it should be noted that the color of fruits and vegetables reflects predominant pigmented phytochemicals, and considering the colors in selection of these food groups provide a wide range of nutraceuticals. In Table 1, some phytochemical-rich fruits and vegetables, their bioactive compounds and favorable effects on diabetic related conditions are reviewed. Studies showed that tomato and its by products, as main sources of lycopene, β-carotene, flavonoids and other bioactive components, could attenuate blood pressure and dyslipidemia, decrease cardiovascular risk factors and enhance antioxidant defense system; other sources of lycopene and carotenoids such as grapefruit and watermelon have also beneficial properties to regulate lipid and lipoprotein metabolism, blood pressure and vascular function. Anthocyanins-rich fruits including red apple, berries family, grapes, cherries, red cabbage, and pomegranate have mainly hypoglycemic effects (↓ digestion and absorption of dietary carbohydrates, ↓ postprandial glycemic response and ↓ glycosylated hemoglobin) as well as protective properties against oxidative damages (Table 1).

LEGUMES

Legumes (peas, beans, lentils, peanuts) are valuable sources of dietary protein, non-digestible carbohydrates including dietary fiber, resistance starches, oligosaccharides, and bioactive compounds such as functional fatty acids (linoleic acid, α-linolenic acid), isoflavones (daidzein, genistein, glycitein), phenolic acids, saponins, and phytic acid; some polyphenols including pelargonidin, cyanidin, delphinidin, and malvidin are also found in legumes[134,135]. Legumes are considered a component of a healthy diet and there is much evidence showing that regular consumption of legumes has protective effects against obesity, type 2 diabetes, and cardiovascular disease[136]. Legumes may be considered as an important component of a functional-foods based diet for management of type 2 diabetes. α-amylase inhibitory peptides are one of the bioactive compounds in legumes and beans that reduce digestion and absorption of dietary carbohydrates, and modulate postprandial glycemic response; other bioactive peptides of grain legumes including the 7S globulin α chain and conglutin γ have unique properties to regulate lipid metabolism and normalize lipid and lipoprotein levels[137]. Low glycemic index, high fiber and phytochemical content of legumes have made them functional food for diabetic patients.

Lentils (Lens culinaris), the most consumed legume grains, are rich sources of dietary fiber, slowly digestible starch and resistant starch, tannins, β-glucan, functional antioxidant ingredients, a wide range of phenolic acids including gallic acid, proanthcyanidins, prodelphinidin, procyanidins, catechins, epicatechin, kampferol, quercetin, cinapic acid and apigenin[138]. Studies show that bioactive proteins of lentil reduce plasma levels of LDL-C, triglyceride content of the liver, and adipose tissue lipoprotein lipase activity; moreover, polyphenols of lentil could prevent angiotensin Ⅱ-induced hypertension, and pathological changes including vascular remodeling and

Table 1 Bioactive compounds and functional properties of some of favorable fruits and vegetables

Ref.	Possible functional properties in diabetes	Main bioactive components and phytochemicals	Fruits and vegetables
[58-62]	↓ Systolic and diastolic blood pressure ↑ apolipoprotein a1 and HDL-C ↓ LDL oxidation, improve diabetes-induced lipid disorders ↓ cardiovascular risk factors ↓ aldose reductase activity and cataract ↑ antioxidative enzymes activity	Lycopene, β-carotene, flavonoids, anthocyanins, phytoan, phyto flava, quercetin, kampferol	Tomato and its by products
[63-65]	↓ Triglyceride levels, enhance endogenous antioxidant defense system, regulation of appetite	Lycopene, pectin, naringin, hesperidin	Grapefruit
[66-69]	↑ Nitric oxide biosynthesis, improve endothelial function ↓ blood pressure ↑ plasma arginine levels and consequently ↓ insulin resistance and adipocyte size	Lycopene, carotenoids, cytrolin	Watermelon
[70-73]	↓ Absorption of dietary carbohydrate ↓ postprandial glycemia, improve pancreatic β-cell function ↓ free radical generation ↓ lipid peroxidation ↑ plasma total antioxidant capacity, prevent vascular damage, improve dyslipidemia	Soluble fiber, quercetin, catechins, epicatechin, *P*-cumaric acid, chlorogenic acid, gallic acid, phlordizin, procyanidins	Red apple, apple peel, apple and its by products
[74-81]	Glycemic control, inhibit α-glucosidase and α-amylase activity ↓ digestion and absorption of dietary carbohydrates ↓ insulin resistance, improve dyslipidemia ↓ postprandial oxidative stress ↓ lipid peroxidation ↑ plasma total antioxidant capacity ↓ systolic blood pressure ↑ antioxidative enzymes activity ↑ adipocytes lipolysis ↓ inflammatory processes, modulation of peroxisome proliferator-activated receptors	Anthocyanins, tannins, ellagitanins, α-carotene, β-carotene, lutein, delphinidins, pelargonidins, ciyanidins, catechins, hydroxy-cinnamic acid	Berries; cranberry, blackberry, black raspberry, blueberry, red raspberry, strawberries
[82-86]	Protective effects on vascular system ↓ platelet hyperactivity and aggregation ↓ cardiovascular diseases ↓ oxidative damage ↓ rennin-angiotensin activity ↑ production of nitric oxide ↓ blood pressure ↑ bone-marrow-derived endothelial progenitor cells	Anthocyanins, resveratrol	Grapes, grape by products
[87-91]	↓ Hyperglycemia ↓ HbA1c, improve lipid disorders, anti-inflammatory properties (inhibit cyclooxygenase) ↓ abdominal fat ↓ microalbuminuria, improve metabolic syndrome and fatty liver features ↓ oxidative stress ↓ production of cytokines, induction of PPARγ ↓ diabetic neuropathy	Anthocyanins, quercetin, hydroxy-cinnamic acid, carotenoids, melatonin, phenolic acids, gallic acid, lutein, xanthine, β-carotene	Cherries
[92-95]	↓ Hyperglycemia, attenuate hyperglycemia-induced metabolic disorders ↓ lipid peroxidation, induction of gluthathione reductase, glutathione peroxidase, superoxide dismutase, delay progression of nephropathy ↓ inflammatory processes, improve dyslipidemia	Isothiocyanates, anthocyanins (red cabbage), carotenoids, lutein, β-carotene	Cabbage, Cauliflower
[96-100]	↓ Hyperglycemia ↑ endothelial nitric oxide synthase activity, inhibit angiotensin converting enzyme ↓ blood pressure, improve vascular function ↓ cholesterol and atherogenic lipids ↓ lipid peroxidation ↓ progression of atherosclerosis ↑ plasma total antioxidant capacity, modulate activation of PPARγ and nuclear factor κB ↑ activity of paraxonase 1 and HDL-C levels ↓ serum resistin levels and ameliorate obesity-induced insulin resistance	Anthocyanins, tannins, catechins, gallocatechins, punicalagin acid, ellagic acid, gallic acid, oleanolic acid, ursolic acid, uallic acid	Pomegranate and its by products, pomegranate peel and seeds

[101-105]	↓ Hyperglycemia, induce insulin secretion from β-cell ↓ blood pressure, inhibit enzyme involved in cholesterol biosynthesis, improve dyslipidemia, prevent atherosclerosis ↓ lipid peroxidation ↓ platelet hyperactivity and aggregation, regulate glycolysis, gluconeogenesis and carbohydrate metabolism pathways ↑ insulin sensitivity	Allyl sulfors, flavonoids, quercetin, dihydroflavonols, anthocyanins (red onion)	Garlic, onions
[106-111]	↓ Endothelial macrophage activation ↓ hyperactivity and aggregation of platelet, improve vascular function ↓ oxidative stress and inhibit stress-sensitive signaling pathways ↓ digestion of dietary lipids, improve dyslipidemia ↓ pro-inflammatory cytokines ↓ lipid peroxidation	Lutein, xanthine, α-cryptoxanthin, β-cryptoxanthin, naringenin, hesperidin, β-carotene, phytosterols	Citrus fruits
[112-113]	↓ Free radical generation and lipid peroxidation, binding to bile acids ↑ cholesterol excretion, improve lipid profile ↑ plasma total antioxidant capacity	Lutein, betaine, violaxanthine, opioid peptides (rubisculins), *P*-cumaric acid, ferulic acid	Spinach
[114-115]	Improve glycemic and insulinemic response ↓ systemic inflammation ↓ cardiovascular disease risk factors	Carotenoids, pectin, oleic and linolenic acids	Pumpkin
[116]	Improve hyperglycemia and dyslipidemia ↑ adiponectin, antioxidant and anti-inflammatory effect	Fiber, polyphenols, chlorogenic acid, flavonoids, anthocyanins	Plums
[117-119]	Improve dyslipidemia, anti-inflammatory properties ↓ lipid peroxidation ↑ plasma total antioxidant capacity	Soluble fiber (pectin), α-carotene, β-carotene lutein, phenolic acids, stilbenes	Carrots
[120-122]	Inhibit α-amylase ↓ postprandial glycemia ↑ glycogen synthesis, improve dyslipidemia ↓ lipid peroxidation, protective effect against diabetic nephropathy	Carotenoids, quercetin, kampferol, gallic acid, caffeic acid, catechins, tannins, mangiferin	Mango
[123-127]	Regulate carbohydrate metabolism (↑ glucokinase and glucose-6-phosphate dehydrogenase activity ↓ glucose-6-phosphatase activity) ↓ lipid peroxidation ↓ protein carbonylation ↑ antioxidant enzyme activity, improve metabolic syndrome features ↑ insulin sensitivity ↓carbohydrate absorption ↓ plasma free fatty acid	Anthocyanins, alkaloid compounds (berberine, oxycontin)	Barberry
[128-131]	Protective effects against diabetic neuropathy ↓ lipid peroxidation, induce antioxidant enzymes, protect liver and kidney against oxidative damage	Dietary fiber, polyphenols, acid cinnamic, melatonin	Date fruit
[132-133]	Improve lipid and lipoprotein metabolism ↑ insulin sensitivity ↓ blood pressure	Dietary fiber, pectin, flavonoids, gallic acid, chlorogenic acid, catechins, anthocyanins	Figs

PPARγ: Peroxisome proliferator-activated receptor γ.

vascular fibrosis[139,140].

Beans are also other important legume grains in the human diet with high content of fiber, phytate, ω_3 fatty acids, antioxidants, phenolic compounds. The hypoglycemic effect of beans (*via* inhibition of α-amylase and β-glucosidase activity) has been reported as being similar to those of anti-diabetic drugs[141-143]. Including beans (pinto, dark red kidney, black beans) in diet planning for type 2 diabetic patients effectively helps weight management, attenuates postprandial glycemic response, and improves dyslipidemia[144-146].

Soybean, a rich source of unique phytoesterogens (genistein, daidzein, glycitein), is another important functional food which has been considered in diabetes; the isoflavones and bioactive peptides of soybean have favorable effects on glycemic control and insulin sensitivity, dyslipidemia, and kidney function[147-149]. It seems that the anti-diabetic effects of soybean mainly occur through interaction with estrogen receptors (ERs); studies show that soy isoflavones selectively bind to both α and β estrogen receptors; ERα is considered as key modulator of glucose and lipid metabolism, and regulate insulin biosynthesis and secretion as well as pancreatic β-cell survival[150]. Soy protein could induce insulin sensitivity and improve lipid homeostasis *via* activation of peroxisome proliferator-activated receptor and liver X receptors, and inhibition of the sterol regulatory element binding protein-1c[151]. Regular consumption of soy products could help diabetic patients in the management of dyslipidemia[152]. Soy protein and isoflavones decrease

production of atherogenic apolipoproteins such as apo B, increase biosynthesis of HDL-C, induce LDL-C receptors, increase biosynthesis and excretion of bile acids, decrease gastrointestinal absorption of steroids, induce favorable changes in hormonal status, including the insulin to glucagon ratio, and thyroid hormones which lead to improvement of dyslipidemia[153,154]. Recently two bioactive peptides, identified in glycinin (a main soy protein), have unique hypolipidemic properties. These peptides inhibit 3-hydroxy-3methyl glutaryl CoA reductase, key enzyme involved in cholesterol biosynthesis. β-conglycinin, another main soy bioactive protein with anti-atherogenic properties *via* regulation of lipogenesis, decease liver lipogenic enzyme activity, inhibits fatty acid biosynthesis in liver, and facilitates fatty acid β-oxidation; other biological activities of soy peptides include antioxidant, anti-inflammatory, and hypotensive effect[155].

Another feature of soybean and soy products as well as other legumes which may highlight them as main part of a functional foods-based diet, is their established effectiveness in weigh management; since the overweight and obesity are the common problems in diabetic patients and main contributors in development of insulin resistance, benefit from anti-obesity properties of legumes is considered another key approach in these patients. Thermogenic effects, induction of satiety through some important appetite regulatory gut peptides, mediation in gene expression and secretion of key adipocytokines such as leptin and adiponectin, as well as inhibitory effects on proliferation and differentiation of adipocytes are some of the mechanisms that could explain the role of legumes on weight management[140,156-159]. In conclusion, considering the potential benefits of legumes and its by products, regular consumption of these functional foods may be an effective strategy for management of various aspects of type 2 diabetes.

NUTS

Based on current evidence, nuts may play a protective effect against cardiovascular disease risk factors. Almonds, pistachios, walnuts and hazelnuts are commonly used nuts; these functional foods are considered as rich sources of high-biological value proteins, bioactive peptides, functional fatty acids (mono and poly unsaturated fatty acids), fiber, phytosterols, polyphenols, tocopherols and other antioxidant vitamins; the antioxidative effect of nuts mainly is related to a high content of α and γ tocopherol, phenolic acids, melatonin, oleic acid and selenium, while the anti-inflammatory effect is related to ellagic acid, α-linolenic acid and magnesium[160,161].

Most current evidence reveals that consumption of nuts in type 2 diabetic patients other than improving the overall diet quality also has beneficial effects on postprandial glycemic response following high-carbohydrate meals, attenuates postprandial oxidative stress and inflammatory processes, normalizes lipid and lipoprotein levels and decreases lipid atherogenicity, and improves insulin resistance[162,163]. Moreover, habitual intake of nuts could help to effectively manage weight especially in diabetic patients; the anti-obesity effects of nuts investigated in some studies may be attributed to thermogenic effects, induction of satiety, decreased dietary fat absorption, and increased fat excretion; bioactive components of nuts also modulate regulatory appetite neurotransmitters and adipose tissue metabolism, as well as decrease proliferation and differentiation of adipocytes, inhibit lipogenesis and induce fatty acid β-oxidation[164,165]. Studies show that consumption of nuts effectively decreases serum levels of high-sensitivity C-reactive protein; a well measure of systemic low-grade inflammation, interleukin 6 (a potent pro-inflammatory cytokine) and fibrinogen while increase plasma concentration of adiponectin, a potent anti-inflammatory cytokine released from adipose tissue; dietary patterns, high in nuts, were also related to lower levels of soluble inflammatory and cardiovascular risk markers including intercellular adhesion molecule 1 and vascular cell adhesion molecule 1[166,167]. Another beneficial effect of nuts which is important especially in diabetic patients is favorably influence on endothelial function; high content of L-arginine, a main precursor of nitric oxide, as well as antioxidants and polyphenols could contribute to this effect[161].

In conclusion, it seems that a diet enriched with nuts may be an effective strategy to improve glycemic control and prevent cardiovascular disease in type 2 diabetic patients.

OTHER BENEFICIAL FUNCTIONAL FOODS AND BIOACTIVE COMPONENTS FOR DIABETIC PATIENTS

Although there are a large number of natural foods, nutraceuticals or bioactive components that could be considered as functional ingredients and have beneficial effects for diabetes management, addressing all these issues is beyond the scope of this article. Table 2 shows some of these potential functional foods including dairy products and probiotics, fish meat, green tea, spices are presented.

CONCLUSION

Type 2 diabetes is a complicated metabolic disorder with both short- and long-term undesirable complications as well as various pathogenic conditions including dyslipidemia, vascular dysfunction, oxidative stress, sub-clinical inflammation, and altered signaling pathways. Ineffectiveness of the current medical treatments in management of long-term diabetes complications confirms that other complementary approaches are required; the use of functional foods and bioactive compounds is one of these new approaches. Functional foods and their bioactive compounds could attenuate carbohydrate metabolism and hyperglycemia, improve pancreatic β-cell function and insulin secretion as well as insulin resistance, regulate lipid and lipoprotein metabolism and adipose tissue metabolism, modulate oxidative/antioxidative balance and

Table 2 Bioactive compounds and functional properties of some of favorable functional foods

Ref.	Possible functional properties in diabetes	Main bioactive components and nutraceuticals	Functional foods
[168-179]	Improve the features of metabolic syndrome, modulate gut microbiota, regulate satiety and food intake ↑ adiponectin, modulate adipocytokines, induce thermogenesis, lipolysis and β-oxidation ↑ dietary fat excretion ↓ adiposity and body weight ↓ oxidative stress and inflammatory markers, hypo-lipidemic and anti-thrombotic effects ↑ insulin sensitivity, modulate immune responses in diabetic patients ↑ total antioxidant capacity ↓ lipid peroxidation ↓ HbA1c	Calcium, vitamin B, bioactive proteins such as casein and whey, immunoglobulines, bioactive peptides (α- and β-lactorphines, lactoferrin, lactoferricin, α-lactalbumin, β-lactoglobulin, growth factors), conjugated linoleic acids, lactic acid bacteria and bifidobacteria	Dairy products and probiotics
[180-185]	Improve hypertriglyceridemia and hypertension ↓ cardiovascular disease ↓ insulin resistance and inflammation, improve glycemic management ↓ proteinuria ↓ oxidative stress, inhibit lipogenesis and induce lipolysis, induce PPARα and PPARβ ↓ adiposity and weight management ↑ thermogenesis and energy expenditure, inhibit angiotensin converting enzyme and modulate blood pressure	Bioactive peptides, antioxidant compounds, ω_3 fatty acids (docosahexaenoic acid, eicosapentaenoic acid), selenium, taurine	Fish and seafood
[186-189]	Regulate cholesterol metabolism ↓ LDL oxidation, protect vascular endothelium against atherogenesis, inhibit platelet aggregation ↓ atherosclerosis development ↓ pro-inflammatory cytokines, activate PPARγ, improve sub-clinical inflammation	Oleic acid, ω_3 fatty acids, Flavonoids, cinnamic acid, benzoic acid, lignans, cumaric acid, ferulic acid, tocopherols, carotenoids, oleuropein, oleocanthal	Olive oil
[190-193]	Promote endogenous antioxidant defense system, induce superoxide dismutase and catalase ↓ lipid peroxidation, improve glycemic control ↑ insulin sensitivity ↓ gluconeogenesis ↑ glycogen content ↓ glycation of collagen and fibrosis, protect cardiac muscle, regulate lipid metabolism as well as adipose tissue metabolism, inhibit lipogenic enzymes ↓ satiety ↑ thermogenesis ↓ proliferation and differentiation of adipocytes ↓ pro-inflammatory cytokines ↓ monocyte chemotactic protein-1	Polyphenols, phenolic acids, catechins, epigallocatechin-3-gallat, chlorophyll, carotenoids, pectin, plant sterols	Green tea
[194-196]	↑ Iinsulin sensitivity, improve peripheral uptake of glucose, increase glycolysis and gluconeogenesis, hypoglycemic and hypolipidemic effects, antioxidant and anti-inflammatory properties	Cinnamaldehyde, cinnamic acid, coumarin, catechins, epicatechin, procyanidins B-2	Cinnamon
[197-199]	Inhibit enzymes involved in inflammation including cyclooxygenase-2, lipoxygenase, and nuclear factor κB, inhibit α-glucosidase and α-amylase activity ↓ postprandial glycemic response ↓ proteinuria, activate PPARγ and regulate carbohydrate and lipid metabolism, prevent diabetic cataract	Curcuminoids, stigmasterol, β-sitosterol, 2-hydroxy methyl anthraquinone, bioactive peptide turmerin	Turmeric
[200-203]	Attenuate oxidative stress, protective effects against oxidative damage ↓ serum creatinine and urea, improve dyslipidemia ↓ atherogenic lipoprotein levels ↓ lipid peroxidation in renal tissue, inhibit α-glucosidase activity ↓ carbohydrate digestion and absorption, protect liver against diabetes-induced oxidative damage	Tannins, flavonoids, anthocyanins, phenolic acid, gallic acid	Sumac

PPAR: Peroxisome proliferator-activated receptor.

inflammatory processes, improve weight management and prevent micro and macro vascular complications.

Considering the beneficial properties of functional foods, it seems that diet planning based on these healthy foods may be considered an effective strategy for management of various aspects of diabetes and promotion of health in diabetic patients.

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REFERENCES

1 **Santaguida PL**, Balion C, Hunt D, Morrison K, Gerstein H, Raina P, Booker L, Yazdi H. Diagnosis, prognosis, and treatment of impaired glucose tolerance and impaired fasting glucose. *Evid Rep Technol Assess* (Summ) 2005; (**128**): 1-11 [PMID: 16194123]

2 **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]

3 **Spranger J**, Kroke A, Möhlig M, Hoffmann K, Bergmann MM, Ristow M, Boeing H, Pfeiffer AF. Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. *Diabetes* 2003; **52**: 812-817 [PMID: 12606524 DOI: 10.2337/diabetes.52.3.812]

4 **Bekyarova GY**, Ivanova DG, Madjova VH. Molecular mechanisms associating oxidative stress with endothelial dysfunction in the development of various vascular complications in diabetes mellitus. *Folia Med* (Plovdiv) 2007; **49**: 13-19 [PMID: 18504928]

5 **Gadi R**, Samaha FF. Dyslipidemia in type 2 diabetes mellitus. *Curr Diab Rep* 2007; **7**: 228-234 [PMID: 17547840 DOI: 10.1007/s11892-007-0036-0]

6 **Constantino MI**, Molyneaux L, Limacher-Gisler F, Al-Saeed A, Luo C, Wu T, Twigg SM, Yue DK, Wong J. Long-term complications and mortality in young-onset diabetes: type 2 diabetes is more hazardous and lethal than type 1 diabetes. *Diabetes Care* 2013; **36**: 3863-3869 [PMID: 23846814]

7 **Lloyd A**, Sawyer W, Hopkinson P. Impact of long-term complications on quality of life in patients with type 2 diabetes not using insulin. *Value Health* 2001; **4**: 392-400 [PMID: 11705130 DOI: 10.1046/j.1524-4733.2001.45029.x]

8 **Bantle JP**, Wylie-Rosett J, Albright AL, Apovian CM, Clark NG, Franz MJ, Hoogwerf BJ, Lichtenstein AH, Mayer-Davis E, Mooradian AD, Wheeler ML. Nutrition recommendations and interventions for diabetes: a position statement of the American Diabetes Association. *Diabetes Care* 2008; **31** Suppl **1**: S61-S78 [PMID: 18165339 DOI: 10.2337/dc08-S061]

9 **Dämon S**, Schätzer M, Höfler J, Tomasec G, Hoppichler F. Nutrition and diabetes mellitus: an overview of the current evidence. *Wien Med Wochenschr* 2011; **161**: 282-288 [PMID: 21442215 DOI: 10.1007/s10354-011-0888-4]

10 **Perera PK**, Li Y. Functional herbal food ingredients used in type 2 diabetes mellitus. *Pharmacogn Rev* 2012; **6**: 37-45 [PMID: 22654403 DOI: 10.4103/0973-7847.95863]

11 **Bahadoran Z**, Mirmiran P, Hosseinpanah F, Hedayati M, Hosseinpour-Niazi S, Azizi F. Broccoli sprouts reduce oxidative stress in type 2 diabetes: a randomized double-blind clinical trial. *Eur J Clin Nutr* 2011; **65**: 972-977 [PMID: 21559038 DOI: 10.1038/ejcn.2011.59]

12 **Bahadoran Z**, Mirmiran P, Hosseinpanah F, Rajab A, Asghari G, Azizi F. Broccoli sprouts powder could improve serum triglyceride and oxidized LDL/LDL-cholesterol ratio in type 2 diabetic patients: a randomized double-blind placebo-controlled clinical trial. *Diabetes Res Clin Pract* 2012; **96**: 348-354 [PMID: 22325157 DOI: 10.1016/j.diabres.2012.01.009]

13 **Bahadoran Z**, Tohidi M, Nazeri P, Mehran M, Azizi F, Mirmiran P. Effect of broccoli sprouts on insulin resistance in type 2 diabetic patients: a randomized double-blind clinical trial. *Int J Food Sci Nutr* 2012; **63**: 767-771 [PMID: 22537070 DOI: 10.3109/09637486.2012.665043]

14 **Kris-Etherton PM**, Lichtenstein AH, Howard BV, Steinberg D, Witztum JL. Antioxidant vitamin supplements and cardiovascular disease. *Circulation* 2004; **110**: 637-641 [PMID: 15289389 DOI: 10.1161/01.CIR.0000137822.39831.F1]

15 **Ballali S**, Lanciai F. Functional food and diabetes: a natural way in diabetes prevention? *Int J Food Sci Nutr* 2012; **63 Suppl 1**: 51-61 [PMID: 22107597 DOI: 10.3109/09637486.2011.637487]

16 **Gregori D**, Gafare CE. Multifunctional food: medical evidence and methodological notes on substantiating health claims. *Int J Food Sci Nutr* 2012; **63 Suppl 1**: 29-36 [PMID: 22289058 DOI: 10.3109/09637486.2011.653553]

17 **Rudkowska I**. Functional foods for health: focus on diabetes. *Maturitas* 2009; **62**: 263-269 [PMID: 19233576 DOI: 10.1016/j.maturitas.2009.01.011]

18 **Bahadoran Z**, Mirmiran P, Azizi F. Dietary polyphenols as potential nutraceuticals in management of diabetes: a review. *J Diabetes Metab Disord* 2013; **12**: 43 [PMID: 23938049 DOI: 10.1186/2251-6581-12-43]

19 **Bahadoran Z**, Mirmiran P, Azizi F. Potential efficacy of broccoli sprouts as a unique supplement for management of type 2 diabetes and its complications. *J Med Food* 2013; **16**: 375-382 [PMID: 23631497 DOI: 10.1089/jmf.2012.2559]

20 **Laaksonen DE**, Toppinen LK, Juntunen KS, Autio K, Liukkonen KH, Poutanen KS, Niskanen L, Mykkänen HM. Dietary carbohydrate modification enhances insulin secretion in persons with the metabolic syndrome. *Am J Clin Nutr* 2005; **82**: 1218-1227 [PMID: 16332654]

21 **Kallio P**, Kolehmainen M, Laaksonen DE, Kekäläinen J, Salopuro T, Sivenius K, Pulkkinen L, Mykkänen HM, Niskanen L, Uusitupa M, Poutanen KS. Dietary carbohydrate modification induces alterations in gene expression in abdominal subcutaneous adipose tissue in persons with the metabolic syndrome: the FUNGENUT Study. *Am J Clin Nutr* 2007; **85**: 1417-1427 [PMID: 17490981]

22 **Borneo R**, León AE. Whole grain cereals: functional components and health benefits. *Food Funct* 2012; **3**: 110-119 [PMID: 22134555 DOI: 10.1039/c1fo10165j]

23 **Ye EQ**, Chacko SA, Chou EL, Kugizaki M, Liu S. Greater whole-grain intake is associated with lower risk of type 2 diabetes, cardiovascular disease, and weight gain. *J Nutr* 2012; **142**: 1304-1313 [PMID: 22649266 DOI: 10.3945/jn.111.155325]

24 **Venn BJ**, Mann JI. Cereal grains, legumes and diabetes. *Eur J Clin Nutr* 2004; **58**: 1443-1461 [PMID: 15162131 DOI: 10.1038/sj.ejcn.1601995]

25 **Okarter N**, Liu RH. Health benefits of whole grain phytochemicals. *Crit Rev Food Sci Nutr* 2010; **50**: 193-208 [PMID: 20301011 DOI: 10.1080/10408390802248734]

26 **Rosén LA**, Silva LO, Andersson UK, Holm C, Ostman EM, Björck IM. Endosperm and whole grain rye breads are characterized by low post-prandial insulin response and a beneficial blood glucose profile. *Nutr J* 2009; **8**: 42 [PMID: 19781071 DOI: 10.1186/1475-2891-8-42]

27 **Shen RL**, Cai FL, Dong JL, Hu XZ. Hypoglycemic effects and biochemical mechanisms of oat products on streptozotocin-induced diabetic mice. *J Agric Food Chem* 2011; **59**: 8895-8900 [PMID: 21749041 DOI: 10.1021/jf200678q]

28 **Martínez I**, Lattimer JM, Hubach KL, Case JA, Yang J, Weber CG, Louk JA, Rose DJ, Kyureghian G, Peterson DA, Haub MD, Walter J. Gut microbiome composition is linked to whole grain-induced immunological improvements. *ISME J* 2013; **7**: 269-280 [PMID: 23038174 DOI: 10.1038/ismej.2012.104]

29 **Andersson A**, Tengblad S, Karlström B, Kamal-Eldin A, Landberg R, Basu S, Aman P, Vessby B. Whole-grain foods do not affect insulin sensitivity or markers of lipid peroxidation and inflammation in healthy, moderately overweight subjects. *J Nutr* 2007; **137**: 1401-1407 [PMID: 17513398]

30 **He M**, van Dam RM, Rimm E, Hu FB, Qi L. Whole-grain, cereal fiber, bran, and germ intake and the risks of all-cause and cardiovascular disease-specific mortality among women with type 2 diabetes mellitus. *Circulation* 2010;

121: 2162-2168 [PMID: 20458012 DOI: 10.1161/CIRCULATIONAHA.109.907360]
31 **Anderson JW**. Whole grains protect against atherosclerotic cardiovascular disease. *Proc Nutr Soc* 2003; **62**: 135-142 [PMID: 12740068 DOI: 10.1079/PNS2002222]
32 **Rosén LA**, Ostman EM, Björck IM. Effects of cereal breakfasts on postprandial glucose, appetite regulation and voluntary energy intake at a subsequent standardized lunch; focusing on rye products. *Nutr J* 2011; **10**: 7 [PMID: 21247415 DOI: 10.1186/1475-2891-10-7]
33 **Sadiq Butt M**, Tahir-Nadeem M, Khan MK, Shabir R, Butt MS. Oat: unique among the cereals. *Eur J Nutr* 2008; **47**: 68-79 [PMID: 18301937 DOI: 10.1007/s00394-008-0698-7]
34 **VandenLangenberg GM**, Brady WE, Nebeling LC, Block G, Forman M, Bowen PE, Stacewicz-Sapuntzakis M, Mares-Perlman JA. Influence of using different sources of carotenoid data in epidemiologic studies. *J Am Diet Assoc* 1996; **96**: 1271-1275 [PMID: 8948389 DOI: 10.1016/S0002-8223(96)00329-X]
35 **Tapola N**, Karvonen H, Niskanen L, Mikola M, Sarkkinen E. Glycemic responses of oat bran products in type 2 diabetic patients. *Nutr Metab Cardiovasc Dis* 2005; **15**: 255-261 [PMID: 16054549 DOI: 10.1016/j.numecd.2004.09.003]
36 **Bays H**, Frestedt JL, Bell M, Williams C, Kolberg L, Schmelzer W, Anderson JW. Reduced viscosity Barley β-Glucan versus placebo: a randomized controlled trial of the effects on insulin sensitivity for individuals at risk for diabetes mellitus. *Nutr Metab* (Lond) 2011; **8**: 58 [PMID: 21846371 DOI: 10.1186/1743-7075-8-58]
37 **Sakuma M**, Yamanaka-Okumura H, Naniwa Y, Matsumoto D, Tsunematsu M, Yamamoto H, Taketani Y, Takeda E. Dose-dependent effects of barley cooked with white rice on postprandial glucose and desacyl ghrelin levels. *J Clin Biochem Nutr* 2009; **44**: 151-159 [PMID: 19308269 DOI: 10.3164/jcbn.08-232]
38 **Gremmel H**, Konrad RM. [Postoperative results in alloplastic closure of diaphragmatic defects]. *Fortschr Geb Rontgenstr Nuklearmed* 1964; **100**: 703-710 [PMID: 14252728]
39 **Kofuji K**, Aoki A, Tsubaki K, Konishi M, Isobe T, Murata Y. Antioxidant Activity of β-Glucan. *ISRN Pharm* 2012; **2012**: 125864 [PMID: 22500243]
40 **Brockman DA**, Chen X, Gallaher DD. Consumption of a high β-glucan barley flour improves glucose control and fatty liver and increases muscle acylcarnitines in the Zucker diabetic fatty rat. *Eur J Nutr* 2013; **52**: 1743-1753 [PMID: 23229409]
41 **Whent M**, Huang H, Xie Z, Lutterodt H, Yu L, Fuerst EP, Morris CF, Yu LL, Luthria D. Phytochemical composition, anti-inflammatory, and antiproliferative activity of whole wheat flour. *J Agric Food Chem* 2012; **60**: 2129-2135 [PMID: 22321109 DOI: 10.1021/jf203807w]
42 **Stevenson L**, Phillips F, O'Sullivan K, Walton J. Wheat bran: its composition and benefits to health, a European perspective. *Int J Food Sci Nutr* 2012; **63**: 1001-1013 [PMID: 22716911 DOI: 10.3109/09637486.2012.687366]
43 **Ross AB**, Kamal-Eldin A, Aman P. Dietary alkylresorcinols: absorption, bioactivities, and possible use as biomarkers of whole-grain wheat- and rye-rich foods. *Nutr Rev* 2004; **62**: 81-95 [PMID: 15098855 DOI: 10.1111/j.1753-4887.2004.tb00029.x]
44 **Brandolini A**, Hidalgo A. Wheat germ: not only a by-product. *Int J Food Sci Nutr* 2012; **63** Suppl 1: 71-74 [PMID: 22077851 DOI: 10.3109/09637486.2011.633898]
45 **Iyer A**, Brown L. Fermented wheat germ extract (avemar) in the treatment of cardiac remodeling and metabolic symptoms in rats. *Evid Based Complement Alternat Med* 2011; **2011**: 508957 [PMID: 19622599]
46 **Callegaro Mda D**, Tirapegui J. [Comparison of the nutritional value between brown rice and white rice]. *Arq Gastroenterol* 1996; **33**: 225-231 [PMID: 9302338]
47 **Shimabukuro M**, Higa M, Kinjo R, Yamakawa K, Tanaka H, Kozuka C, Yabiku K, Taira S, Sata M, Masuzaki H. Effects of the brown rice diet on visceral obesity and endothelial function: the BRAVO study. *Br J Nutr* 2014; **111**: 310-320 [PMID: 23930929]
48 **Zhang P**, Tang H, Chen K, Chen Y, Xu D. Biological variations of hematologic parameters determined by UniCel DxH 800 hematology analyzer. *Arch Pathol Lab Med* 2013; **137**: 1106-1110 [PMID: 23899069 DOI: 10.1186/1472-6882-13-198]
49 **Kozuka C**, Yabiku K, Takayama C, Matsushita M, Shimabukuro M. Natural food science based novel approach toward prevention and treatment of obesity and type 2 diabetes: recent studies on brown rice and γ-oryzanol. *Obes Res Clin Pract* 2013; **7**: e165-e172 [PMID: 23697584 DOI: 10.1016/j.orcp.2013.02.003]
50 **Torimitsu M**, Nagase R, Yanagi M, Homma M, Sasai Y, Ito Y, Hayamizu K, Nonaka S, Hosono T, Kise M, Seki T, Ariga T. Replacing white rice with pre-germinated brown rice mildly ameliorates hyperglycemia and imbalance of adipocytokine levels in type 2 diabetes model rats. *J Nutr Sci Vitaminol* (Tokyo) 2010; **56**: 287-292 [PMID: 21228498 DOI: 10.3177/jnsv.56.287]
51 **Usuki S**, Tsai YY, Morikawa K, Nonaka S, Okuhara Y, Kise M, Yu RK. IGF-1 induction by acylated steryl β-glucosides found in a pre-germinated brown rice diet reduces oxidative stress in streptozotocin-induced diabetes. *PLoS One* 2011; **6**: e28693 [PMID: 22194889 DOI: 10.1371/journal.pone.0028693]
52 **Cheng HH**, Huang HY, Chen YY, Huang CL, Chang CJ, Chen HL, Lai MH. Ameliorative effects of stabilized rice bran on type 2 diabetes patients. *Ann Nutr Metab* 2010; **56**: 45-51 [PMID: 20016147 DOI: 10.1159/000265850]
53 **Heber D**. Vegetables, fruits and phytoestrogens in the prevention of diseases. *J Postgrad Med* 2004; **50**: 145-149 [PMID: 15235216]
54 **Hegde SV**, Adhikari P, M N, D'Souza V. Effect of daily supplementation of fruits on oxidative stress indices and glycaemic status in type 2 diabetes mellitus. *Complement Ther Clin Pract* 2013; **19**: 97-100 [PMID: 23561067 DOI: 10.1016/j.ctcp.2012.12.002]
55 **Tanaka S**, Yoshimura Y, Kawasaki R, Kamada C, Tanaka S, Horikawa C, Ohashi Y, Araki A, Ito H, Akanuma Y, Yamada N, Yamashita H, Sone H. Fruit intake and incident diabetic retinopathy with type 2 diabetes. *Epidemiology* 2013; **24**: 204-211 [PMID: 23348071 DOI: 10.1097/EDE.0b013e318281725e]
56 **Takahashi K**, Kamada C, Yoshimura H, Okumura R, Iimuro S, Ohashi Y, Araki A, Umegaki H, Sakurai T, Yoshimura Y, Ito H. Effects of total and green vegetable intakes on glycated hemoglobin A1c and triglycerides in elderly patients with type 2 diabetes mellitus: the Japanese Elderly Intervention Trial. *Geriatr Gerontol Int* 2012; **12** Suppl 1: 50-58 [PMID: 22435940 DOI: 10.1111/j.1447-0594.2011.00812.x]
57 **Chan HT**, Yiu KH, Wong CY, Li SW, Tam S, Tse HF. Increased dietary fruit intake was associated with lower burden of carotid atherosclerosis in Chinese patients with Type 2 diabetes mellitus. *Diabet Med* 2013; **30**: 100-108 [PMID: 22913415 DOI: 10.1111/j.1464-5491.2012.03764.x]
58 **Ali MM**, Agha FG. Amelioration of streptozotocin-induced diabetes mellitus, oxidative stress and dyslipidemia in rats by tomato extract lycopene. *Scand J Clin Lab Invest* 2009; **69**: 371-379 [PMID: 19148834 DOI: 10.1080/00365510802658473]
59 **Canene-Adams K**, Campbell JK, Zaripheh S, Jeffery EH, Erdman JW. The tomato as a functional food. *J Nutr* 2005; **135**: 1226-1230 [PMID: 15867308]
60 **Pollack A**, Oren P, Stark AH, Eisner Z, Nyska A, Madar Z. Cataract development in sand and galactosemic rats fed a natural tomato extract. *J Agric Food Chem* 1999; **47**: 5122-5126 [PMID: 10606583 DOI: 10.1021/jf9900231]
61 **Shidfar F**, Froghifar N, Vafa M, Rajab A, Hosseini S, Shidfar S, Gohari M. The effects of tomato consumption on serum

glucose, apolipoprotein B, apolipoprotein A-I, homocysteine and blood pressure in type 2 diabetic patients. *Int J Food Sci Nutr* 2011; **62**: 289-294 [PMID: 21138408 DOI: 10.3109/09637486.2010.529072]
62 **Upritchard JE**, Sutherland WH, Mann JI. Effect of supplementation with tomato juice, vitamin E, and vitamin C on LDL oxidation and products of inflammatory activity in type 2 diabetes. *Diabetes Care* 2000; **23**: 733-738 [PMID: 10840987 DOI: 10.2337/diacare.23.6.733]
63 **Goldwasser J**, Cohen PY, Yang E, Balaguer P, Yarmush ML, Nahmias Y. Transcriptional regulation of human and rat hepatic lipid metabolism by the grapefruit flavonoid naringenin: role of PPARalpha, PPARgamma and LXRalpha. *PLoS One* 2010; **5**: e12399 [PMID: 20811644 DOI: 10.1371/journal.pone.0012399]
64 **Gorinstein S**, Caspi A, Libman I, Lerner HT, Huang D, Leontowicz H, Leontowicz M, Tashma Z, Katrich E, Feng S, Trakhtenberg S. Red grapefruit positively influences serum triglyceride level in patients suffering from coronary atherosclerosis: studies in vitro and in humans. *J Agric Food Chem* 2006; **54**: 1887-1892 [PMID: 16506849 DOI: 10.1021/jf058171g]
65 **Owira PM**, Ojewole JA. The grapefruit: an old wine in a new glass? Metabolic and cardiovascular perspectives. *Cardiovasc J Afr* 2010; **21**: 280-285 [PMID: 20972517]
66 **Wu G**, Collins JK, Perkins-Veazie P, Siddiq M, Dolan KD, Kelly KA, Heaps CL, Meininger CJ. Dietary supplementation with watermelon pomace juice enhances arginine availability and ameliorates the metabolic syndrome in Zucker diabetic fatty rats. *J Nutr* 2007; **137**: 2680-2685 [PMID: 18029483]
67 **Figueroa A**, Sanchez-Gonzalez MA, Perkins-Veazie PM, Arjmandi BH. Effects of watermelon supplementation on aortic blood pressure and wave reflection in individuals with prehypertension: a pilot study. *Am J Hypertens* 2011; **24**: 40-44 [PMID: 20616787 DOI: 10.1038/ajh.2010.142]
68 **Fatema K**, Habib B, Afza N, Ali L. Glycemic, non-esterified fatty acid (NEFA) and insulinemic responses to watermelon and apple in type 2 diabetic subjects. *Asia Pac J Clin Nutr* 2003; **12** Suppl: S53 [PMID: 15023673]
69 **Martins MJ**, Negrão MR, Azevedo I. Watermelon: the value of higher plasma arginine concentrations. *Nutrition* 2007; **23**: 517 [PMID: 17467236 DOI: 10.1016/j.nut.2007.03.007]
70 **Setorki M**, Asgary S, Eidi A, Rohani AH, Esmaeil N. Effects of apple juice on risk factors of lipid profile, inflammation and coagulation, endothelial markers and atherosclerotic lesions in high cholesterolemic rabbits. *Lipids Health Dis* 2009; **8**: 39 [PMID: 19804641 DOI: 10.1186/1476-511X-8-39]
71 **Boyer J**, Liu RH. Apple phytochemicals and their health benefits. *Nutr J* 2004; **3**: 5 [PMID: 15140261]
72 **Chai SC**, Hooshmand S, Saadat RL, Payton ME, Brummel-Smith K, Arjmandi BH. Daily apple versus dried plum: impact on cardiovascular disease risk factors in postmenopausal women. *J Acad Nutr Diet* 2012; **112**: 1158-1168 [PMID: 22818725 DOI: 10.1016/j.jand.2012.05.005]
73 **Hyson DA**. A comprehensive review of apples and apple components and their relationship to human health. *Adv Nutr* 2011; **2**: 408-420 [PMID: 22332082 DOI: 10.3945/an.111.000513]
74 **Kaume L**, Howard LR, Devareddy L. The blackberry fruit: a review on its composition and chemistry, metabolism and bioavailability, and health benefits. *J Agric Food Chem* 2012; **60**: 5716-5727 [PMID: 22082199]
75 **Seymour EM**, Tanone II, Urcuyo-Llanes DE, Lewis SK, Kirakosyan A, Kondoleon MG, Kaufman PB, Bolling SF. Blueberry intake alters skeletal muscle and adipose tissue peroxisome proliferator-activated receptor activity and reduces insulin resistance in obese rats. *J Med Food* 2011; **14**: 1511-1518 [PMID: 21861718 DOI: 10.1089/jmf.2010.0292]
76 **Suh JH**, Romain C, González-Barrio R, Cristol JP, Teissèdre PL, Crozier A, Rouanet JM. Raspberry juice consumption, oxidative stress and reduction of atherosclerosis risk factors in hypercholesterolemic golden Syrian hamsters. *Food Funct* 2011; **2**: 400-405 [PMID: 21894327 DOI: 10.1039/c1fo10047e]
77 **Apostolidis E**, Kwon YI, Shetty K. Potential of cranberry-based herbal synergies for diabetes and hypertension management. *Asia Pac J Clin Nutr* 2006; **15**: 433-441 [PMID: 16837438]
78 **Basu A**, Fu DX, Wilkinson M, Simmons B, Wu M, Betts NM, Du M, Lyons TJ. Strawberries decrease atherosclerotic markers in subjects with metabolic syndrome. *Nutr Res* 2010; **30**: 462-469 [PMID: 20797478 DOI: 10.1016/j.nutres.2010.06.016]
79 **Chen CF**, Li YD, Xu Z. [Chemical principles and bioactivities of blueberry]. *Yaoxue Xuebao* 2010; **45**: 422-429 [PMID: 21355205]
80 **Morimoto C**, Satoh Y, Hara M, Inoue S, Tsujita T, Okuda H. Anti-obese action of raspberry ketone. *Life Sci* 2005; **77**: 194-204 [PMID: 15862604 DOI: 10.1016/j.lfs.2004.12.029]
81 **Basu A**, Rhone M, Lyons TJ. Berries: emerging impact on cardiovascular health. *Nutr Rev* 2010; **68**: 168-177 [PMID: 20384847 DOI: 10.1111/j.1753-4887.2010.00273.x]
82 **Castilla P**, Echarri R, Dávalos A, Cerrato F, Ortega H, Teruel JL, Lucas MF, Gómez-Coronado D, Ortuño J, Lasunción MA. Concentrated red grape juice exerts antioxidant, hypolipidemic, and antiinflammatory effects in both hemodialysis patients and healthy subjects. *Am J Clin Nutr* 2006; **84**: 252-262 [PMID: 16825703]
83 **Felice F**, Zambito Y, Di Colo G, D'Onofrio C, Fausto C, Balbarini A, Di Stefano R. Red grape skin and seeds polyphenols: Evidence of their protective effects on endothelial progenitor cells and improvement of their intestinal absorption. *Eur J Pharm Biopharm* 2012; **80**: 176-184 [PMID: 21971368 DOI: 10.1016/j.ejpb.2011.09.002]
84 **Gollücke AP**, Ribeiro DA. Use of grape polyphenols for promoting human health: a review of patents. *Recent Pat Food Nutr Agric* 2012; **4**: 26-30 [PMID: 22316271 DOI: 10.2174/1876142911204010026]
85 **Leifert WR**, Abeywardena MY. Cardioprotective actions of grape polyphenols. *Nutr Res* 2008; **28**: 729-737 [PMID: 19083481 DOI: 10.1016/j.nutres.2008.08.007]
86 **Vislocky LM**, Fernandez ML. Biomedical effects of grape products. *Nutr Rev* 2010; **68**: 656-670 [PMID: 20961296 DOI: 10.1111/j.1753-4887.2010.00335.x]
87 **Kim DO**, Heo HJ, Kim YJ, Yang HS, Lee CY. Sweet and sour cherry phenolics and their protective effects on neuronal cells. *J Agric Food Chem* 2005; **53**: 9921-9927 [PMID: 16366675 DOI: 10.1021/jf0518599]
88 **Seymour EM**, Lewis SK, Urcuyo-Llanes DE, Tanone II, Kirakosyan A, Kaufman PB, Bolling SF. Regular tart cherry intake alters abdominal adiposity, adipose gene transcription, and inflammation in obesity-prone rats fed a high fat diet. *J Med Food* 2009; **12**: 935-942 [PMID: 19857054 DOI: 10.1089/jmf.2008.0270]
89 **Traustadóttir T**, Davies SS, Stock AA, Su Y, Heward CB, Roberts LJ, Harman SM. Tart cherry juice decreases oxidative stress in healthy older men and women. *J Nutr* 2009; **139**: 1896-1900 [PMID: 19692530 DOI: 10.3945/jn.109.111716]
90 **Ataie-Jafari A**, Hosseini S, Karimi F. Effects of sour cherry juice on blood glucose and some cardiovascular risk factors improvements in diabetic women: A pilot study. *Nutr Food Sci* 2008; **38**: 355-360 [DOI: 10.1108/00346650810891414]
91 **Zhou Z**, Nair MG, Claycombe KJ. Synergistic inhibition of interleukin-6 production in adipose stem cells by tart cherry anthocyanins and atorvastatin. *Phytomedicine* 2012; **19**: 878-881 [PMID: 22703874 DOI: 10.1016/j.phymed.2012.05.001]
92 **Islam MS**, Choi H. Antidiabetic effect of Korean traditional Baechu (Chinese cabbage) kimchi in a type 2 diabetes model of rats. *J Med Food* 2009; **12**: 292-297 [PMID: 19459728 DOI: 10.1089/jmf.2008.0181]
93 **Kataya HA**, Hamza AA. Red Cabbage (Brassica oleracea) Ameliorates Diabetic Nephropathy in Rats. *Evid Based Complement Alternat Med* 2008; **5**: 281-287 [PMID: 18830445 DOI: 10.1093/ecam/nem029]

94 **Sankhari JM**, Thounaojam MC, Jadeja RN, Devkar RV, Ramachandran AV. Anthocyanin-rich red cabbage (Brassica oleracea L.) extract attenuates cardiac and hepatic oxidative stress in rats fed an atherogenic diet. *J Sci Food Agric* 2012; **92**: 1688-1693 [PMID: 22228433 DOI: 10.1002/jsfa.5532]

95 **Dinkova-Kostova AT**, Kostov RV. Glucosinolates and isothiocyanates in health and disease. *Trends Mol Med* 2012; **18**: 337-347 [PMID: 22578879 DOI: 10.1016/j.molmed.2012.04.003]

96 **Aviram M**, Dornfeld L. Pomegranate juice consumption inhibits serum angiotensin converting enzyme activity and reduces systolic blood pressure. *Atherosclerosis* 2001; **158**: 195-198 [PMID: 11500191 DOI: 10.1016/S0021-9150(01)00412-9]

97 **Basu A**, Penugonda K. Pomegranate juice: a heart-healthy fruit juice. *Nutr Rev* 2009; **67**: 49-56 [PMID: 19146506 DOI: 10.1111/j.1753-4887.2008.00133.x]

98 **Banihani S**, Swedan S, Alguraan Z. Pomegranate and type 2 diabetes. *Nutr Res* 2013; **33**: 341-348 [DOI: 10.1016/j.nutres.2013.03.003]

99 **Mathew AS**, Capel-Williams GM, Berry SE, Hall WL. Acute effects of pomegranate extract on postprandial lipaemia, vascular function and blood pressure. *Plant Foods Hum Nutr* 2012; **67**: 351-357 [PMID: 23093401 DOI: 10.1007/s11130-012-0318-9]

100 **Medjakovic S**, Jungbauer A. Pomegranate: a fruit that ameliorates metabolic syndrome. *Food Funct* 2013; **4**: 19-39 [PMID: 23060097 DOI: 10.1039/c2fo30034f]

101 **Slimestad R**, Fossen T, Vågen IM. Onions: a source of unique dietary flavonoids. *J Agric Food Chem* 2007; **55**: 10067-10080 [PMID: 17997520 DOI: 10.1021/jf0712503]

102 **Taj Eldin IM**, Ahmed EM, Elwahab H M A. Preliminary Study of the Clinical Hypoglycemic Effects of Allium cepa (Red Onion) in Type 1 and Type 2 Diabetic Patients. *Environ Health Insights* 2010; **4**: 71-77 [PMID: 21079693]

103 **Gabler NK**, Osrowska E, Imsic M, Eagling DR, Jois M, Tatham BG, Dunshea FR. Dietary onion intake as part of a typical high fat diet improves indices of cardiovascular health using the mixed sex pig model. *Plant Foods Hum Nutr* 2006; **61**: 179-185 [PMID: 17123162 DOI: 10.1007/s11130-006-0030-8]

104 **Ashraf R**, Aamir K, Shaikh AR, Ahmed T. Effects of garlic on dyslipidemia in patients with type 2 diabetes mellitus. *J Ayub Med Coll Abbottabad* 2005; **17**: 60-64 [PMID: 16320801]

105 **Ashraf R**, Khan RA, Ashraf I. Garlic (Allium sativum) supplementation with standard antidiabetic agent provides better diabetic control in type 2 diabetes patients. *Pak J Pharm Sci* 2011; **24**: 565-570 [PMID: 21959822]

106 **Yamada T**, Hayasaka S, Shibata Y, Ojima T, Saegusa T, Gotoh T, Ishikawa S, Nakamura Y, Kayaba K. Frequency of citrus fruit intake is associated with the incidence of cardiovascular disease: the Jichi Medical School cohort study. *J Epidemiol* 2011; **21**: 169-175 [PMID: 21389640 DOI: 10.2188/jea.JE20100084]

107 **Ramful D**, Tarnus E, Rondeau P, Robert Da Silva C, Bahorun T, Bourdon E. Citrus Fruit Extracts Reduce Advanced Glycation End Products (AGEs)- and H2O2-Induced Oxidative Stress in Human Adipocytes. *J Agric Food Chem* 2010; **58**: 11119-11129 [PMID: 20882960]

108 **Mulvihill EE**, Huff MW. Citrus flavonoids and the prevention of atherosclerosis. *Cardiovasc Hematol Disord Drug Targets* 2012; **12**: 84-91 [PMID: 23030447 DOI: 10.2174/1871529X11202020084]

109 **Buscemi S**, Rosafio G, Arcoleo G, Mattina A, Canino B, Montana M, Verga S, Rini G. Effects of red orange juice intake on endothelial function and inflammatory markers in adult subjects with increased cardiovascular risk. *Am J Clin Nutr* 2012; **95**: 1089-1095 [PMID: 22492368 DOI: 10.3945/ajcn.111.031088]

110 **Chanet A**, Milenkovic D, Manach C, Mazur A, Morand C. Citrus flavanones: what is their role in cardiovascular protection? *J Agric Food Chem* 2012; **60**: 8809-8822 [PMID: 22574825 DOI: 10.1021/jf300669s]

111 **Assini JM**, Mulvihill EE, Huff MW. Citrus flavonoids and lipid metabolism. *Curr Opin Lipidol* 2013; **24**: 34-40 [PMID: 23254473 DOI: 10.1097/MOL.0b013e32835c07fd]

112 **Bergman M**, Varshavsky L, Gottlieb HE, Grossman S. The antioxidant activity of aqueous spinach extract: chemical identification of active fractions. *Phytochemistry* 2001; **58**: 143-152 [PMID: 11524124 DOI: 10.1016/S0031-9422(01)00137-6]

113 **Moser B**, Szekeres T, Bieglmayer C, Wagner KH, Mišík M, Kundi M, Zakerska O, Nersesyan A, Kager N, Zahrl J, Hoelzl C, Ehrlich V, Knasmueller S. Impact of spinach consumption on DNA stability in peripheral lymphocytes and on biochemical blood parameters: results of a human intervention trial. *Eur J Nutr* 2011; **50**: 587-594 [PMID: 21384253 DOI: 10.1007/s00394-011-0167-6]

114 **Yadav M**, Jain S, Tomar R, Prasad GB, Yadav H. Medicinal and biological potential of pumpkin: an updated review. *Nutr Res Rev* 2010; **23**: 184-190 [PMID: 21110905 DOI: 10.1017/S0954422410000107]

115 **Yoshinari O**, Sato H, Igarashi K. Anti-diabetic effects of pumpkin and its components, trigonelline and nicotinic acid, on Goto-Kakizaki rats. *Biosci Biotechnol Biochem* 2009; **73**: 1033-1041 [PMID: 19420712 DOI: 10.1271/bbb.80805]

116 **Utsunomiya H**, Yamakawa T, Kamei J, Kadonosono K, Tanaka S. Anti-hyperglycemic effects of plum in a rat model of obesity and type 2 diabetes, Wistar fatty rat. *Biomed Res* 2005; **26**: 193-200 [PMID: 16295695 DOI: 10.2220/biomedres.26.193]

117 **Potter AS**, Foroudi S, Stamatikos A, Patil BS, Deyhim F. Drinking carrot juice increases total antioxidant status and decreases lipid peroxidation in adults. *Nutr J* 2011; **10**: 96 [PMID: 21943297 DOI: 10.1186/1475-2891-10-96]

118 **Ramezani A**, Tahbaz F, Rasooli Sh, Rashidkhani B, Gharavi Noori A, Moslemi N, Hedayati M. Effects of ß -carotene-fortified carrot juice on the lipid profile in type-2 diabetes patients. *Iranian Journal of Nutrition Sciences and Food Technology* 2010; **5**: 57-66

119 **Metzger BT**, Barnes DM, Reed JD. Purple carrot (Daucus carota L.) polyacetylenes decrease lipopolysaccharide-induced expression of inflammatory proteins in macrophage and endothelial cells. *J Agric Food Chem* 2008; **56**: 3554-3560 [PMID: 18433135 DOI: 10.1021/jf073494t]

120 **Lucas EA**, Li W, Peterson SK, Brown A, Kuvibidila S, Perkins-Veazie P, Clarke SL, Smith BJ. Mango modulates body fat and plasma glucose and lipids in mice fed a high-fat diet. *Br J Nutr* 2011; **106**: 1495-1505 [PMID: 21733317 DOI: 10.1017/S0007114511002066]

121 **Masibo M**, He Q. Mango Bioactive Compounds and Related Nutraceutical Properties: A Review. *Food Rev* 2009; **25**: 346-370 [DOI: 10.1080/87559120903153524]

122 **Li X**, Cui X, Sun X, Li X, Zhu Q, Li W. Mangiferin prevents diabetic nephropathy progression in streptozotocin-induced diabetic rats. *Phytother Res* 2010; **24**: 893-899 [PMID: 19960420]

123 **Zhou J**, Zhou S, Tang J, Zhang K, Guang L, Huang Y, Xu Y, Ying Y, Zhang L, Li D. Protective effect of berberine on beta cells in streptozotocin- and high-carbohydrate/high-fat diet-induced diabetic rats. *Eur J Pharmacol* 2009; **606**: 262-268 [PMID: 19374872]

124 **Fatehi M**, Saleh TM, Fatehi-Hassanabad Z, Farrokhfal K, Jafarzadeh M, Davodi S. A pharmacological study on Berberis vulgaris fruit extract. *J Ethnopharmacol* 2005; **102**: 46-52 [PMID: 15993555 DOI: 10.1016/j.jep.2005.05.019]

125 **Fatehi-Hassanabad Z**, Jafarzadeh M, Tarhini A, Fatehi M. The antihypertensive and vasodilator effects of aqueous extract from Berberis vulgaris fruit on hypertensive rats. *Phytother Res* 2005; **19**: 222-225 [PMID: 15934023 DOI: 10.1002/ptr.1661]

126 **Gao CR**, Zhang JQ, Huang QL. [Experimental study on berberin raised insulin sensitivity in insulin resistance rat mod-

els]. *Zhongguo Zhongxiyi Jiehe Zazhi* 1997; **17**: 162-164 [PMID: 9863084]
127 **Yin J**, Hu R, Chen M, Tang J, Li F, Yang Y, Chen J. Effects of berberine on glucose metabolism in vitro. *Metabolism* 2002; **51**: 1439-1443 [PMID: 12404195 DOI: 10.1053/meta.2002.34715]
128 **Al-Farsi MA**, Lee CY. Nutritional and functional properties of dates: a review. *Crit Rev Food Sci Nutr* 2008; **48**: 877-887 [PMID: 18949591 DOI: 10.1080/10408390701724264]
129 **Saafi EB**, Louedi M, Elfeki A, Zakhama A, Najjar MF, Hammami M, Achour L. Protective effect of date palm fruit extract (Phoenix dactylifera L.) on dimethoate induced-oxidative stress in rat liver. *Exp Toxicol Pathol* 2011; **63**: 433-441 [PMID: 20359872 DOI: 10.1016/j.etp.2010.03.002]
130 **Vayalil PK**. Date fruits (Phoenix dactylifera Linn): an emerging medicinal food. *Crit Rev Food Sci Nutr* 2012; **52**: 249-271 [PMID: 22214443 DOI: 10.1080/10408398.2010.499824]
131 **Zangiabadi N**, Asadi-Shekaari M, Sheibani V, Jafari M, Shabani M, Asadi AR, Tajadini H, Jarahi M. Date fruit extract is a neuroprotective agent in diabetic peripheral neuropathy in streptozotocin-induced diabetic rats: a multimodal analysis. *Oxid Med Cell Longev* 2011; **2011**: 976948 [PMID: 22191015]
132 **Slatnar A**, Klancar U, Stampar F, Veberic R. Effect of drying of figs (Ficus carica L.) on the contents of sugars, organic acids, and phenolic compounds. *J Agric Food Chem* 2011; **59**: 11696-11702 [PMID: 21958361 DOI: 10.1021/jf202707y]
133 **Josef B**, Raj S. Pharmacognostic and phytochemical properties of Ficus carica Linn: An overview. *Int J Pharma Tech Res* 2011; **3**: 8-12
134 **Duranti M**. Grain legume proteins and nutraceutical properties. *Fitoterapia* 2006; **77**: 67-82 [PMID: 16406359 DOI: 10.1016/j.fitote.2005.11.008]
135 **Madar Z**, Stark AH. New legume sources as therapeutic agents. *Br J Nutr* 2002; **88** Suppl 3: S287-S292 [PMID: 12498629 DOI: 10.1079/BJN2002719]
136 **Flight I**, Clifton P. Cereal grains and legumes in the prevention of coronary heart disease and stroke: a review of the literature. *Eur J Clin Nutr* 2006; **60**: 1145-1159 [PMID: 16670693 DOI: 10.1038/sj.ejcn.1602435]
137 **López-Amorós ML**, Hernández T, Estrella I. Estrella I. Effect of germination on legume phenolic compounds and their antioxidant activity. *J Food Comp Anal* 2006; **19**: 277-283 [DOI: 10.1016/j.jfca.2004.06.012]
138 **Zou Y**, Chang SK, Gu Y, Qian SY. Antioxidant activity and phenolic compositions of lentil (Lens culinaris var. Morton) extract and its fractions. *J Agric Food Chem* 2011; **59**: 2268-2276 [PMID: 21332205 DOI: 10.1021/jf104640k]
139 **Boualga A**, Prost J, Taleb-Senouci D, Krouf D, Kharoubi O, Lamri-Senhadji M, Belleville J, Bouchenak M. Purified chickpea or lentil proteins impair VLDL metabolism and lipoprotein lipase activity in epididymal fat, but not in muscle, compared to casein, in growing rats. *Eur J Nutr* 2009; **48**: 162-169 [PMID: 19165521 DOI: 10.1007/s00394-009-0777-4]
140 **Yao F**, Sun C, Chang SK. Lentil polyphenol extract prevents angiotensin II-induced hypertension, vascular remodelling and perivascular fibrosis. *Food Funct* 2012; **3**: 127-133 [PMID: 22159297 DOI: 10.1039/c1fo10142k]
141 **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]
142 **Yao Y**, Cheng XZ, Wang LX, Wang SH, Ren G. Major Phenolic Compounds, Antioxidant Capacity and Antidiabetic Potential of Rice Bean (Vigna umbellata L.) in China. *Int J Mol Sci* 2012; **13**: 2707-2716 [PMID: 22489119 DOI: 10.3390/ijms13032707]
143 **Helmstädter A**. Beans and diabetes: Phaseolus vulgaris preparations as antihyperglycemic agents. *J Med Food* 2010; **13**: 251-254 [PMID: 20132042 DOI: 10.1089/jmf.2009.0002]
144 **Geil PB**, Anderson JW. Nutrition and health implications of dry beans: a review. *J Am Coll Nutr* 1994; **13**: 549-558 [PMID: 7706585 DOI: 10.1080/07315724.1994.10718446]
145 **Preuss HG**. Bean amylase inhibitor and other carbohydrate absorption blockers: effects on diabesity and general health. *J Am Coll Nutr* 2009; **28**: 266-276 [PMID: 20150600 DOI: 10.1080/07315724.2009.10719781]
146 **Thompson SV**, Winham DM, Hutchins AM. Bean and rice meals reduce postprandial glycemic response in adults with type 2 diabetes: a cross-over study. *Nutr J* 2012; **11**: 23 [PMID: 22494488 DOI: 10.1186/1475-2891-11-23]
147 **Anderson JW**, Bush HM. Soy protein effects on serum lipoproteins: a quality assessment and meta-analysis of randomized, controlled studies. *J Am Coll Nutr* 2011; **30**: 79-91 [PMID: 21730216 DOI: 10.1080/07315724.2011.10719947]
148 **Azadbakht L**, Shakerhosseini R, Atabak S, Jamshidian M, Mehrabi Y, Esmaill-Zadeh A. Beneficiary effect of dietary soy protein on lowering plasma levels of lipid and improving kidney function in type II diabetes with nephropathy. *Eur J Clin Nutr* 2003; **57**: 1292-1294 [PMID: 14506491 DOI: 10.1038/sj.ejcn.1601688]
149 **Bhathena SJ**, Velasquez MT. Beneficial role of dietary phytoestrogens in obesity and diabetes. *Am J Clin Nutr* 2002; **76**: 1191-1201 [PMID: 12450882]
150 **Gilbert ER**, Liu D. Anti-diabetic functions of soy isoflavone genistein: mechanisms underlying its effects on pancreatic β-cell function. *Food Funct* 2013; **4**: 200-212 [PMID: 23160185 DOI: 10.1039/c2fo30199g]
151 **Ronis MJ**, Chen Y, Badeaux J, Badger TM. Dietary soy protein isolate attenuates metabolic syndrome in rats via effects on PPAR, LXR, and SREBP signaling. *J Nutr* 2009; **139**: 1431-1438 [PMID: 19515742 DOI: 10.3945/jn.109.107029]
152 **Yang B**, Chen Y, Xu T, Yu Y, Huang T, Hu X, Li D. Systematic review and meta-analysis of soy products consumption in patients with type 2 diabetes mellitus. *Asia Pac J Clin Nutr* 2011; **20**: 593-602 [PMID: 22094845]
153 **Borradaile NM**, de Dreu LE, Wilcox LJ, Edwards JY, Huff MW. Soya phytoestrogens, genistein and daidzein, decrease apolipoprotein B secretion from HepG2 cells through multiple mechanisms. *Biochem J* 2002; **366**: 531-539 [PMID: 12030847 DOI: 10.1042/BJ20020046]
154 **Potter SM**. Overview of proposed mechanisms for the hypocholesterolemic effect of soy. *J Nutr* 1995; **125**: 606S-611S [PMID: 7884541]
155 **Liu CF**, Pan TM. Beneficial Effects of Bioactive Peptides Derived from Soybean on Human Health and Their Production by Genetic Engineering. *Soybean and health* 2011: 311-329
156 **Frigolet ME**, Torres N, Uribe-Figueroa L, Rangel C, Jimenez-Sanchez G, Tovar AR. White adipose tissue genome wide-expression profiling and adipocyte metabolic functions after soy protein consumption in rats. *J Nutr Biochem* 2011; **22**: 118-129 [PMID: 20471815 DOI: 10.1016/j.jnutbio.2009.12.006]
157 **Cheik NC**, Rossi EA, Guerra RL, Tenório NM, Oller do Nascimento CM, Viana FP, Manzoni MS, Carlos IZ, Leão da Silva P, Vendramini RC, Dâmaso AR. Effects of a ferment soy product on the adipocyte area reduction and dyslipidemia control in hypercholesterolemic adult male rats. *Lipids Health Dis* 2008; **7**: 50 [PMID: 19087298 DOI: 10.1186/1476-511X-7-50]
158 **Zhang Z**, Lanza E, Ross AC, Albert PS, Colburn NH, Rovine MJ, Bagshaw D, Ulbrecht JS, Hartman TJ. A high-legume low-glycemic index diet reduces fasting plasma leptin in middle-aged insulin-resistant and -sensitive men. *Eur J Clin Nutr* 2011; **65**: 415-418 [PMID: 21206508 DOI: 10.1038/ejcn.2010.273]
159 **Segura R**, Javierre C, Lizarraga MA, Ros E. Other relevant components of nuts: phytosterols, folate and minerals. *Br J Nutr* 2006; **96** Suppl 2: S36-S44 [PMID: 17125532 DOI: 10.1017/BJN20061862]
160 **Kendall CW**, Esfahani A, Truan J, Srichaikul K, Jenkins DJ. Health benefits of nuts in prevention and management of diabetes. *Asia Pac J Clin Nutr* 2010; **19**: 110-116 [PMID:

20199995]
161 **Ros E**. Nuts and novel biomarkers of cardiovascular disease. *Am J Clin Nutr* 2009; **89**: 1649S-1656S [PMID: 19321561 DOI: 10.3945/ajcn.2009.26736R]
162 **Jenkins DJ**, Hu FB, Tapsell LC, Josse AR, Kendall CW. Possible benefit of nuts in type 2 diabetes. *J Nutr* 2008; **138**: 1752S-1756S [PMID: 18716181]
163 **Li TY**, Brennan AM, Wedick NM, Mantzoros C, Rifai N, Hu FB. Regular consumption of nuts is associated with a lower risk of cardiovascular disease in women with type 2 diabetes. *J Nutr* 2009; **139**: 1333-1338 [PMID: 19420347 DOI: 10.3945/jn.108.103622]
164 **Tey SL**, Brown R, Gray A, Chisholm A, Delahunty C. Nuts improve diet quality compared to other energy-dense snacks while maintaining body weight. *J Nutr Metab* 2011; **2011**: 357350 [PMID: 21845219]
165 **Mattes RD**, Dreher ML. Nuts and healthy body weight maintenance mechanisms. *Asia Pac J Clin Nutr* 2010; **19**: 137-141 [PMID: 20199999]
166 **Mantzoros CS**, Williams CJ, Manson JE, Meigs JB, Hu FB. Adherence to the Mediterranean dietary pattern is positively associated with plasma adiponectin concentrations in diabetic women. *Am J Clin Nutr* 2006; **84**: 328-335 [PMID: 16895879]
167 **Jiang R**, Jacobs DR, Mayer-Davis E, Szklo M, Herrington D, Jenny NS, Kronmal R, Barr RG. Nut and seed consumption and inflammatory markers in the multi-ethnic study of atherosclerosis. *Am J Epidemiol* 2006; **163**: 222-231 [PMID: 16357111 DOI: 10.1093/aje/kwj033]
168 **Al-Salami H**, Butt G, Fawcett JP, Tucker IG, Golocorbin-Kon S, Mikov M. Probiotic treatment reduces blood glucose levels and increases systemic absorption of gliclazide in diabetic rats. *Eur J Drug Metab Pharmacokinet* 2008; **33**: 101-106 [PMID: 18777945 DOI: 10.1007/BF03191026]
169 **Ataie-Jafari A**, Larijani B, Alavi Majd H, Tahbaz F. Cholesterol-lowering effect of probiotic yogurt in comparison with ordinary yogurt in mildly to moderately hypercholesterolemic subjects. *Ann Nutr Metab* 2009; **54**: 22-27 [PMID: 19229114 DOI: 10.1159/000203284]
170 **Chang BJ**, Park SU, Jang YS, Ko SH, Joo NM, Kim SI, Kim CH, Chang DK. Effect of functional yogurt NY-YP901 in improving the trait of metabolic syndrome. *Eur J Clin Nutr* 2011; **65**: 1250-1255 [PMID: 21697819 DOI: 10.1038/ejcn.2011.115]
171 **Guo C**, Zhang L. [Cholesterol-lowering effects of probiotics--a review]. *Weishengwu Xuebao* 2010; **50**: 1590-1599 [PMID: 21365911]
172 **Guzel-Seydim ZB**, Kok-Tas T, Greene AK, Seydim AC. Review: functional properties of kefir. *Crit Rev Food Sci Nutr* 2011; **51**: 261-268 [PMID: 21390946 DOI: 10.1080/10408390903579029]
173 **Lin MY**, Yen CL. Reactive oxygen species and lipid peroxidation product-scavenging ability of yogurt organisms. *J Dairy Sci* 1999; **82**: 1629-1634 [PMID: 10480088 DOI: 10.3168/jds.S0022-0302(99)75391-9]
174 **Liu S**, Choi HK, Ford E, Song Y, Klevak A, Buring JE, Manson JE. A prospective study of dairy intake and the risk of type 2 diabetes in women. *Diabetes Care* 2006; **29**: 1579-1584 [PMID: 16801582 DOI: 10.2337/dc06-0256]
175 **Lopitz-Otsoa F**, Rementeria A, Elguezabal N, Garaizar J. Kefir: a symbiotic yeasts-bacteria community with alleged healthy capabilities. *Rev Iberoam Micol* 2006; **23**: 67-74 [PMID: 16854180 DOI: 10.1016/S1130-1406(06)70016-X]
176 **Marteau PR**. Probiotics in clinical conditions. *Clin Rev Allergy Immunol* 2002; **22**: 255-273 [PMID: 12043384 DOI: 10.1007/s12016-002-0011-0]
177 **Pfeuffer M**, Schrezenmeir J. Milk and the metabolic syndrome. *Obes Rev* 2007; **8**: 109-118 [PMID: 17300277 DOI: 10.1111/j.1467-789X.2006.00265.x]
178 **Shab-Bidar S**, Neyestani TR, Djazayery A. Efficacy of vitamin D3-fortified-yogurt drink on anthropometric, metabolic, inflammatory and oxidative stress biomarkers according to vitamin D receptor gene polymorphisms in type 2 diabetic patients: a study protocol for a randomized controlled clinical trial. *BMC Endocr Disord* 2011; **11**: 12 [PMID: 21696575 DOI: 10.1186/1472-6823-11-12]
179 **Teruya K**, Yamashita M, Tominaga R, Nagira T, Shim SY, Katakura Y, Tokumaru S, Tokumaru K, Barnes D, Shirahata S. Fermented milk, Kefram-Kefir enhances glucose uptake into insulin-responsive muscle cells. *Cytotechnology* 2002; **40**: 107-116 [PMID: 19003111 DOI: 10.1023/A:1023926407877]
180 **Boukortt FO**, Girard A, Prost JL, Ait-Yahia D, Bouchenak M, Belleville J. Fish protein improves the total antioxidant status of streptozotocin-induced diabetes in spontaneously hypertensive rat. *Med Sci Monit* 2004; **10**: BR397-BR404 [PMID: 15507843]
181 **Farmer A**, Montori V, Dinneen S, Clar C. Fish oil in people with type 2 diabetes mellitus. *Cochrane Database Syst Rev* 2001; (**3**): CD003205 [PMID: 11687050]
182 **Jacques H**, Gascon A, Bergeron N, Lavigne C, Hurley C, Deshaies Y, Moorjani S, Julien P. Role of dietary fish protein in the regulation of plasma lipids. *Can J Cardiol* 1995; **11** Suppl G: 63G-71G [PMID: 7585295]
183 **Lankinen M**, Schwab U, Kolehmainen M, Paananen J, Poutanen K, Mykkänen H, Seppänen-Laakso T, Gylling H, Uusitupa M, Orešič M. Whole grain products, fish and bilberries alter glucose and lipid metabolism in a randomized, controlled trial: the Sysdimet study. *PLoS One* 2011; **6**: e22646 [PMID: 21901116 DOI: 10.1371/journal.pone.0022646]
184 **McEwen B**, Morel-Kopp MC, Tofler G, Ward C. Effect of omega-3 fish oil on cardiovascular risk in diabetes. *Diabetes Educ* 2010; **36**: 565-584 [PMID: 20534874 DOI: 10.1177/0145721710372675]
185 **Pecis M**, de Azevedo MJ, Gross JL. Chicken and fish diet reduces glomerular hyperfiltration in IDDM patients. *Diabetes Care* 1994; **17**: 665-672 [PMID: 7924775 DOI: 10.2337/diacare.17.7.665]
186 **Cicerale S**, Conlan XA, Sinclair AJ, Keast RS. Chemistry and health of olive oil phenolics. *Crit Rev Food Sci Nutr* 2009; **49**: 218-236 [PMID: 19093267 DOI: 10.1080/10408390701856223]
187 **Lucas L**, Russell A, Keast R. Molecular mechanisms of inflammation. Anti-inflammatory benefits of virgin olive oil and the phenolic compound oleocanthal. *Curr Pharm Des* 2011; **17**: 754-768 [PMID: 21443487 DOI: 10.2174/138161211795428911]
188 **Madigan C**, Ryan M, Owens D, Collins P, Tomkin GH. Dietary unsaturated fatty acids in type 2 diabetes: higher levels of postprandial lipoprotein on a linoleic acid-rich sunflower oil diet compared with an oleic acid-rich olive oil diet. *Diabetes Care* 2000; **23**: 1472-1477 [PMID: 11023139 DOI: 10.2337/diacare.23.10.1472]
189 **Wardhana ES**, Datau EA. The role of omega-3 fatty acids contained in olive oil on chronic inflammation. *Acta Med Indones* 2011; **43**: 138-143 [PMID: 21785178]
190 **Babu PV**, Sabitha KE, Srinivasan P, Shyamaladevi CS. Green tea attenuates diabetes induced Maillard-type fluorescence and collagen cross-linking in the heart of streptozotocin diabetic rats. *Pharmacol Res* 2007; **55**: 433-440 [PMID: 17336542 DOI: 10.1016/j.phrs.2007.01.019]
191 **Fiorino P**, Evangelista FS, Santos F, Motter Magri FM, Delorenzi JC, Ginoza M, Farah V. The effects of green tea consumption on cardiometabolic alterations induced by experimental diabetes. *Exp Diabetes Res* 2012; **2012**: 309231 [PMID: 22474420]
192 **Kumar B**, Gupta SK, Nag TC, Srivastava S, Saxena R. Green tea prevents hyperglycemia-induced retinal oxidative stress and inflammation in streptozotocin-induced diabetic rats. *Ophthalmic Res* 2012; **47**: 103-108 [PMID: 21997135]
193 **Kim HM**, Kim J. The effects of green tea on obesity and type 2 diabetes. *Diabetes Metab J* 2013; **37**: 173-175 [PMID: 23807919 DOI: 10.4093/dmj.2013.37.3.173]
194 **Anderson RA**. Chromium and polyphenols from cinnamon

improve insulin sensitivity. *Proc Nutr Soc* 2008; **67**: 48-53 [PMID: 18234131 DOI: 10.1017/S0029665108006010]

195 **Gruenwald J**, Freder J, Armbruester N. Cinnamon and health. *Crit Rev Food Sci Nutr* 2010; **50**: 822-834 [PMID: 20924865 DOI: 10.1080/10408390902773052]

196 **Kirkham S**, Akilen R, Sharma S, Tsiami A. The potential of cinnamon to reduce blood glucose levels in patients with type 2 diabetes and insulin resistance. *Diabetes Obes Metab* 2009; **11**: 1100-1113 [PMID: 19930003 DOI: 10.1111/j.1463-1326.2009.01094.x]

197 **Honda S**, Aoki F, Tanaka H, Kishida H, Nishiyama T, Okada S, Matsumoto I, Abe K, Mae T. Effects of ingested turmeric oleoresin on glucose and lipid metabolisms in obese diabetic mice: a DNA microarray study. *J Agric Food Chem* 2006; **54**: 9055-9062 [PMID: 17117790 DOI: 10.1021/jf061788t]

198 **Khajehdehi P**, Pakfetrat M, Javidnia K, Azad F, Malekmakan L, Nasab MH, Dehghanzadeh G. Oral supplementation of turmeric attenuates proteinuria, transforming growth factor-β and interleukin-8 levels in patients with overt type 2 diabetic nephropathy: a randomized, double-blind and placebo-controlled study. *Scand J Urol Nephrol* 2011; **45**: 365-370 [PMID: 21627399 DOI: 10.3109/00365599.2011.585622]

199 **Lekshmi PC**, Arimboor R, Raghu KG, Menon AN. Turmerin, the antioxidant protein from turmeric (Curcuma longa) exhibits antihyperglycaemic effects. *Nat Prod Res* 2012; **26**: 1654-1658 [PMID: 21972920]

200 **Rayne S**, Mazza G. Biological activities of extracts from sumac (Rhus spp.): a review. *Plant Foods Hum Nutr* 2007; **62**: 165-175 [PMID: 17909971 DOI: 10.1007/s11130-007-0058-4]

201 **Shafiei M**, Nobakht M, Moazzam AA. Lipid-lowering effect of Rhus coriaria L. (sumac) fruit extract in hypercholesterolemic rats. *Pharmazie* 2011; **66**: 988-992 [PMID: 22312707]

202 **Giancarlo S**, Rosa LM, Nadjafi F, Francesco M. Hypoglycaemic activity of two spices extracts: Rhus coriaria L. and Bunium persicum Boiss. *Nat Prod Res* 2006; **20**: 882-886 [PMID: 16753927 DOI: 10.1080/14786410500520186]

203 **Pourahmad J**, Eskandari MR, Shakibaei R, Kamalinejad M. A search for hepatoprotective activity of aqueous extract of Rhus coriaria L. against oxidative stress cytotoxicity. *Food Chem Toxicol* 2010; **48**: 854-858 [PMID: 20036300]

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TOPIC HIGHLIGHT

WJD 5th Anniversary Special Issues (3): Type 1 diabetes

Why do some patients with type 1 diabetes live so long?

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Abstract

While the lifespan of people with type 1 diabetes has increased progressively since the advent of insulin therapy, these patients still experience premature mortality, primarily from cardiovascular disease (CVD). However, a subgroup of those with type 1 diabetes survives well into old age without significant morbidity. It is the purpose of this review to explore the factors which may help in identifying these patients. It might be expected that hyperglycaemia plays a major role in explaining the increased incidence of CVD and mortality of these individuals. However, while a number of publications have associated poor long term glycaemic control with an increase in both all-cause mortality and CVD in those with type 1 diabetes, it is apparent that good glycaemic control alone cannot explain why some patients with type 1 diabetes avoid fatal CVD events. Lipid disorders may occur in those with type 1 diabetes, but the occurrence of elevated high-density lipoprotein-cholesterol is positively associated with longevity in this population. Non-renal hypertension, by itself is a significant risk factor for CVD but if adequately treated does not appear to mitigate against longevity. However, the presence of nephropathy is a major risk factor and its absence after 15-20 years of diabetes appears to be a marker of long-term survival. One of the major factors linked with long-term survival is the absence of features of the metabolic syndrome and more specifically the presence of insulin sensitivity. Genetic factors also play a role, with a family history of longevity and an absence of type 2 diabetes and hypertension in the family being important considerations. There is thus a complex interaction between multiple risk factors in determining which patients with type 1 diabetes are likely to live into older age. However, these patients can often be identified clinically based on a combination of factors as outlined above.

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Key words: Type 1 diabetes; Prognosis; Survival; Coronary artery disease; Cardiovascular disease; Lipids; Metabolic syndrome

Core tip: People with type 1 diabetes are generally assumed to have a shortened lifespan. This contention is supported by a number of epidemiological studies confirming a trend towards premature death, primarily due to cardiovascular disease. However, a subset of type 1 individuals survives for many years, living for over 50 years or more with type 1 diabetes. This review explores the clinical features that are linked to long-term survival in people with type 1 diabetes, allowing identification of these individuals. Recognising these individuals will aid in assessing prognosis, and treating the identified risk factors could improve survival.

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INTRODUCTION

Prior to the discovery of insulin, patients with type 1 diabetes had an expected lifespan of less than 3 years[1].

With the advent of modern therapy, survival has increased progressively. However, those with type 1 diabetes remain with an increased incidence of coronary artery disease (CAD) and mortality compared to the general population. By 1991, reported standard mortality rates for those with type 1 diabetes under the age of 60 years were 9.1 for males and 13.5 for females[2]. Subsequently, a cohort of 23751 patients from the United Kingdom and diagnosed with diabetes under the age of 30 years between 1972 and 1993 were analysed for cardiovascular mortality up to 2000[3]. These results confirmed higher mortality rates at younger ages for those with type 1 diabetes (Figure 1). Of interest, not only are the mortality rates for women with diabetes considerably higher than for women without diabetes, but also higher than for men without diabetes. Soedamah-Muthu *et al*[4], utilizing the United Kingdom General Practice research database, have also confirmed that the risk of cardiovascular disease (CVD) remains high in patients with type 1 diabetes. Typically, patients with type 1 diabetes reach a 10-year risk of fatal CVD of 5% about 10 to 15 years before the general population. Furthermore, incidence rates of CAD in type 1 patients range between 1.2% and 2% per year, *vs* 0.1% and 0.5% in the general population[5]. The incidence of stroke is also increased in type 1 diabetes, with overall standardised incidence ratios being 17.94 for men and 26.11 for women[6].

It is therefore clear, that despite a better understanding and treatment of appropriate risk factors and better general care, those with type 1 diabetes still have a tendency towards a shortened life span, primarily due to premature CVD. Yet a subgroup of individuals with type 1 diabetes survives well into old age in relatively good health. This review explores the factors that may help to identify these patients. This can be done either by identifying a group of long-surviving type 1 patients and analysing any unique clinical or biological features that may be specific to this cohort, or by assessing surrogate endpoints of vascular disease, such as carotid artery Intima-Media Thickness (IMT) measurement or arterial calcification and identifying those who appear to be "protected" from vascular disease.

THE ROLE OF GLYCAEMIC CONTROL

Type 1 diabetes is a condition of "pure" hyperglycaemia. The only abnormality is one of β-cell failure and insulin deficiency in an otherwise "normal" or "healthy" individual. It could therefore be expected that hyperglycaemia might play a major role in explaining the increased incidence of CVD and mortality seen in these individuals. A number of publications have associated poor long-term glycaemic control with an increase in both all-cause mortality and CVD in those with type 1 diabetes. Grauslund *et al*[7] demonstrated a direct relationship between HbA1c and survival. When patients were categorized into quartiles of HbA1c measurements, patients in the highest quartile had a significantly higher risk of all-cause mortality, cardiovascular mortality and ischaemic heart disease

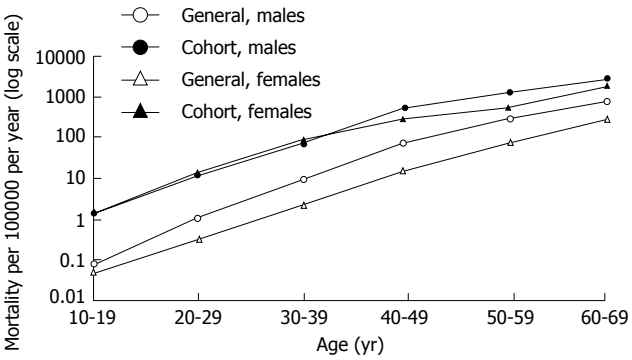

Figure 1 Ischaemic heart disease mortality rates in people with type 1 diabetes *vs* general population (from: Laing *et al*[3]).

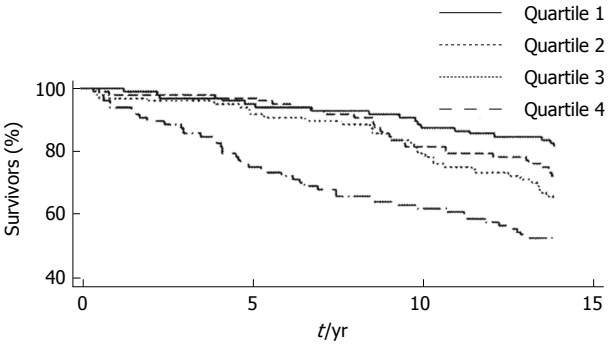

Figure 2 All-cause mortality and the association of glycaemic control (from: Grauslund *et al*[7]).

when compared to patients in the lowest quartile (Figure 2). While at the conclusion of the Diabetes Control and Complications Trial (DCCT) there was no significant difference between the conventional and intensive treatment groups regarding cardiovascular outcomes or death from CVD, the 10-year Epidemiology of Diabetes Interventions and Complications (EDIC) follow-up demonstrated a significant difference between the two groups with regard to both CV outcomes and death. An overall 42% risk reduction was seen in the previously intensively treated group[8]. This sustained effect of improved control in the DCCT years was ascribed to "metabolic memory". Patients followed in the DCCT/EDIC cohort were also submitted to IMT measurements, and it was demonstrated that intensive therapy during the DCCT resulted in decreased progression of IMT six year after the end of the trial[9]. These findings imply that early glycaemic control is an important factor in preventing CVD in type 1 diabetes.

However, good blood glucose levels alone cannot explain why some patients with type 1 diabetes avoid fatal CVD events. In the "Golden Years Cohort" of 400 type 1 patients who survived for over 50 years with diabetes[10], the mean HbA1c was 7.6% (± 1.4), with some of these patients having HbA1c levels as high as 8.5%-9%. None had an HbA1c below 7%. In addition, a number of other publications have shown only a weak correlation between long-term glycaemic control, CVD and mortality. Larsen *et al*[11], performed coronary angiography on 29 asymptomatic patients with a mean duration

of type 1 diabetes of 30.6 years. Of these, 34% had significant coronary artery stenosis. While a significant relationship existed between stenosis and glycaemic control (a 6.1% increase in vessel stenosis for every 1% increase in HbA1c over 18 years), glycaemic control was less significant as a risk factor than the age of the subjects and the effect of elevated serum cholesterol. In another cohort of 125 patients with a mean duration of diabetes of 22 years[12], IMT was compared to an index of lifetime glycaemic exposure. This demonstrated significantly increased IMT only on those at the highest tertile of glycaemic exposure. IMT measurements performed in 148 long-surviving patients with type 1 diabetes (duration > 15 years)[13] showed no significant correlation between HbA1c and IMT, although ordinal logistic regression showed that for every 1% increase in HbA1c, there was a 27% less chance of the IMT falling into the low-risk group (defined as an IMT below 0.6 mm and no plaque). A prospective observational study of a meta-analysis of the relationship between CVD and glycaemic control[14], revealed an only moderate increase in cardiovascular risk with increasing levels of glycated haemoglobin in persons with diabetes mellitus. However, this meta-analysis included patients with both type 1 and type 2 diabetes. The data suggested that there is an increased risk of CVD of 15% for every 1% increase in HbA1c (RR = 1.15; 95%CI: 0.92-1.43).

The evidence therefore suggests that while early good glycaemic control is important in the prevention of CVD and survival, the importance of glycaemic control may diminish as patients survive longer. While glycaemic control is clearly a risk factor for CAD and mortality in type 1 diabetes, this is not the major determinant of survival. Good glycaemic control alone cannot explain why some type 1 patients survive into old age.

LIPIDS IN TYPE 1 DIABETES

Patients with type 1 diabetes may show quantitative lipid disorders. There is a clear relationship between the level of glycaemic control and lipid abnormalities, with an independent correlation between HbA1c and low-density lipoprotein (LDL)-cholesterol, non-high-density lipoprotein (HDL) cholesterol and triglycerides[15]. Abnormal lipid levels are associated with worse cardiovascular outcomes[5]. The lipid profiles of patients with well-controlled type 1 diabetes are very different from those with poor glycaemic control[16], related possibly to the presence of adequate peripheral insulin levels in the better controlled subjects. There are direct metabolic consequences of administering insulin subcutaneously. Peripheral hyperinsulinemia is associated with increased lipoprotein lipase activity[17], which may account for reduced triglyceride levels. In addition, LDL-cholesterol may also be slightly reduced due to decreased very LDL production[18]. The more sensitive the individual is to insulin, the greater is this effect.

As might be expected, Serum LDL-cholesterol and non-HDL-cholesterol levels are positively associated with not only an increase in IMT[9], increased Arterial Stiffness[19] and coronary artery stenosis[11], but also CAD and mortality[5,7,20]. A major factor that appears to be associated with prolonged survival in patients with type 1 diabetes is elevated HDL-cholesterol. HDL levels are often elevated in those with type 1 diabetes. This is more marked with better glycaemic control and may be due to an elevated lipoprotein lipase/hepatic lipase ratio (Increased peripheral lipoprotein lipase activity due to peripheral hyperinsulinemia from subcutaneous insulin administration and normal hepatic lipase activity). Bain *et al*[10] reported a high mean HDL-level in those surviving over 50 years with diabetes (1.84 ± 0.057 mmol/L), and this was associated with lower triglyceride levels (1.49 ± 0.79 mmol/L). In long-surviving type 1 patients, IMT measurements showed a significant inverse association to HDL levels and computed tomography/HDL ratios for all measure of risk (IM thickness and/or plaque)[13]. A number of other studies have supported the protective effects of HDL-cholesterol with regard to CVD[5,7,9,11,20]. In addition to this direct association between HDL-cholesterol and CVD, higher HDL-cholesterol levels may provide protection against the development of albuminuria[21].

Therefore, it can be concluded that in addition to the expected effect of dyslipidaemia (high LDL and non-HDL-cholesterol), HDL-cholesterol itself exerts a significant protective effect on the development of CVD in patients with type 1 diabetes and elevated HDL-cholesterol levels appears to play a major role in longevity in these patients.

BLOOD PRESSURE AS A RISK FACTOR

Hypertension in those with type 1 diabetes is often a manifestation of underlying nephropathy. However, hypertension can also occur as a stand-alone risk factor (non-renal hypertension). A significant positive association between high blood pressure and arterial stiffness in youth with type 1 diabetes was demonstrated in the SEARCH CVD Study[19].

In type 1 diabetes, hypertension without nephropathy has been shown to be a major risk factor for the development of carotid artery plaque [OR = 5.26 ($P < 0.004$)], but the effect of hypertension on IMT was moderate and not significant[13]. In the DCCT/EDIC at 6 years, the presence of hypertension and particularly systolic hypertension was significant, but had less of an effect on IMT than did smoking, lipids or glycaemic control[9]. In the Golden years cohort[10], 29% of the patients were receiving antihypertensive treatment but had nevertheless survived for over 50 years with diabetes.

It therefore appears as though hypertension itself, while a significant risk factor for CVD, if treated does not mitigate against longevity in this population.

MICROVASCULAR DISEASE AS A MARKER OF SURVIVAL

The presence of diabetic nephropathy, microalbuminuria

Table 1 Cox proportional hazard models for risk of cardiovascular disease from nephropathy (from: Grausland *et al*[7])

	All cause mortality	Cardiovascular mortality	IHD
Creatinine > 120 μmol/L	5.1	6.29	4.25
Microalbuminuria	1.32	1.44	1.40
Macroalbuminuria	2.4	2.57	1.77

IHD: Ischaemic heart disease.

or macroalbuminuria is a significant risk factor for CAD, cardiovascular mortality and all cause mortality, and there is a strong independent relationship between albuminuria and CAD (Table 1)[7]. The occurrence of stroke in subjects with type 1 diabetes is also increased by the presence of nephropathy [microalbuminuria: HR = 3.2 (1.9-5.6), macroalbuminuria: HR = 4.9 (2.9-8.2), End Stage Renal Disease: HR = 7.5 (4.2-13.3)][22]. The DCCT/EDIC Study showed a sustained effect of good glycaemic control[23] on the reduction in albumin excretion 7 years after the conclusion of the DCCT study, with an 83% risk reduction in those patients initially treated with intensive therapy, confirming the concept of "metabolic memory". The long-term risk of a reduction in estimated glomerular filtration rate (eGFR) was also shown to be 50% lower among those who were treated early in the course of type 1 diabetes with intensive diabetes therapy than among those treated with conventional diabetes therapy[24]. The development of hypertension was also delayed in the intensively treated group. These effects appeared to be largely mediated by the levels of glycaemia achieved during the DCCT. However, as pointed out by the authors, a long time elapsed between treatment intensification during the DCCT early in the course of the diabetes and the effect on eGFR, and the advantages of improved glycaemic control in persons already with advanced complications may not apply. This further supports the contention that good glycaemic control in the early years of the diabetes may be more important achieved in those who have had the condition for some years.

In type 1 diabetes, the peak incidence of nephropathy occurs between 15 and 20 years after the development of the diabetes[25,26]. Progression from microalbuminuria to overt neuropathy has been shown to reduce from 45% in those with diabetes of less than 15 years, to 26% in those with diabetes of over 15 years duration. By the time someone has had diabetes for over 40 years, it drops to just 4% per year[25]. In this regard, none of the long surviving patient in the "Golden Years cohort"[10] had evidence of overt nephropathy.

It is therefore apparent, that those individuals with type 1 diabetes who are likely to survive, would remain free of any evidence of nephropathy.

No prospective studies in type 1 patients have found a strong independent relationship between retinopathy and CVD or mortality. However, the presence of retinopathy increases the risk of stroke[22]. Severe diabetic retinopathy was common in the "Golden Years Cohort"[10]. Forty-three percent of subjects had had laser therapy and 2% were blind. In relatively long-surviving people with type 1 diabetes, the presence of retinopathy had a significant association with the presence of plaque (OR = 3.65; $P < 0.033$), independent of glycaemic control[13]. However, there was no association between the presence of retinopathy and IMT measurements. It therefore appears as though retinopathy is not a major risk factor for CVD or mortality in those with type 1 diabetes, as opposed to those with type 2 diabetes where the presence of retinopathy may indicate CAD and mortality risk[27].

With regard to peripheral neuropathy, no prospective trials link the presence of neuropathy to either CAD or mortality other than the EURODIAB study, which did detect peripheral and autonomic neuropathy as risk markers for future mortality[20].

TYPE 1 DIABETES AND THE METABOLIC SYNDROME

There is no reason to expect patients with type 1 diabetes to have a lower prevalence of obesity and the metabolic syndrome (MetS) than the general population and a MetS frequency in type 1 patients of over 30% has been reported[28]. A significant relationship exists between mortality and central obesity in those with type 1 diabetes[20] and type 1 subjects with the MetS have been shown to have an increased prevalence of macrovascular disease[29]. The presence of MetS features in patients with type 1 diabetes is associated with risk factors similar to many patients with type 2 diabetes, and the superimposition of the insulin resistance due to obesity or the MetS in a patient who already has type 1 diabetes has been termed "Double diabetes"[30].

Identifying patients with the MetS in the presence of type 1 diabetes is difficult. Of the diagnostic criteria, the presence of dysglycaemia is a foregone conclusion and cannot be used. Hypertension should only be included if it is non-renal as nephropathy-induced hypertension has other implications as outlined above. Quantifying insulin resistance is also difficult and requires a euglycaemic clamp study to document it properly. A derived estimate of glucose disposal rate has been suggested to measure of insulin resistance[31] but this includes the presence of hypertension and waist-hip ratio in the formula and therefore cannot be used in assessing insulin resistance in the context of the MetS, since both of these variables are separate components of the MetS in their own right. Insulin dosage provides a surrogate measurement of insulin resistance in these patients, and in their series of long-surviving type 1 patients, Distiller *et al*[32] arbitrarily chose insulin doses in the top quartile of their series of patients (0.75 U/kg body weight), to be a measure of insulin resistance. In this series, a multiple linear regression analysis showed a significant relationship between waist circumference and insulin dose and carotid artery IMT when corrected for age of onset, current age and duration of diabetes. Interestingly, neither body mass index (BMI) nor HbA1c were significantly associated with carotid artery IMT. Overall, there was a significant

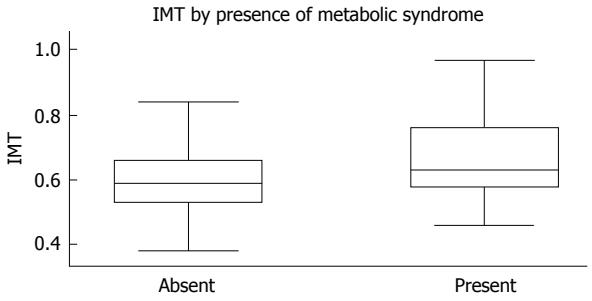

Figure 3 A significant increase in Intima-Media Thickness is seen in patients s with the metabolic syndrome (*P* = 0.003) (adapted from: Distiller *et al*[32]).

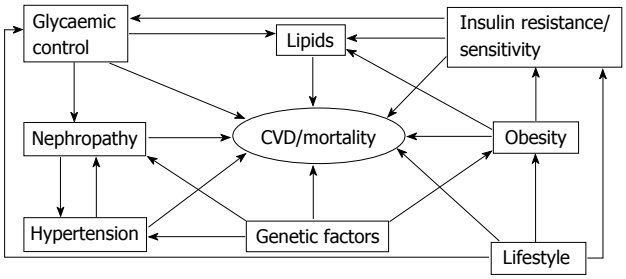

Figure 4 Complex interactions exist between multiple risk factors in determining the outcome for patients with type 1 diabetes. CVD: Cardiovascular disease.

increase in IMT in type 1 subjects with the MetS (Figure 3). A significant association was demonstrated between IMT risk and the number of features of the MetS (P = 0.01). Fifty percent of patients with 0-1 features had low risk IMT, whereas 60% of patients with 3-4 features had high risk IMT measures. This finding was confirmed by the SEARCH CVD Study[16], a longitudinal study of 298 youth with diabetes, where those with the MetS had consistently increased arterial wall stiffness when compared to type 1 patients without the Syndrome and with the same duration of diabetes. This was born out by the "Golden Years Cohort"[10], where the patients were generally on low doses of insulin. The mean daily insulin dose was 37.5 U (± 16.2) (0.52 U/kg body weight), the mean BMI of these long surviving patients was 25 kg/m^2, and HDL-cholesterol was high and triglycerides were low. These features could be considered the antithesis of the MetS.

GENETIC FACTORS

The best predictor of old age is the age one's parents achieved. This adage was supported by the "Golden Years Cohort"[10], where on average, both parents of those surviving 50 years with diabetes lived to over 70 years. Furthermore, a family history of either type 2 diabetes or hypertension has been shown to result in significantly increased IMT in type 1 diabetes subjects[12].

Clearly, a complex interaction exists between multiple risk factors in determining which patients with type 1 diabetes are likely to live into older age (Figure 4). However, these patients can often be identified clinically based on a combination of factors (Table 2).

Table 2 Identifying features of long-surviving patients with type 1 diabetes

Reasonable (not necessarily ideal) glycaemic control
High HDL-cholesterol levels
Low daily insulin requirements ("insulin sensitive")
Normal body weight
Non-smokers
Lower blood pressures
Microalbumin negative after 15-20 yr of diabetes
Family history of longevity

HDL: High density lipoprotein.

CONCLUSION

While the longevity of those with type 1 diabetes has improved considerably over the past century, these patients remain with a reduced life expectancy compared to the non-diabetic population. Nevertheless, a subgroup of these individuals may survive into older age despite their diabetes. Certain clinical and biochemical features can identify these people. This understanding may provide clinicians with further evidence that correction of modifiable risk factors like glycaemic control, blood pressure control, avoidance of excessive weight gain and lipid control is vital in ensuring the ongoing longevity of patients with type 1 diabetes.

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REFERENCES

1 **Gale EAM**. Historical aspects of type 1 diabetes [internet]. Available from: URL: http: //www.diapedia.org/type-1-diabetes-mellitus/2104085134/historical-aspects-of-type-1-diabetes
2 **Moss SE**, Klein R, Klein BE. Cause-specific mortality in a population-based study of diabetes. *Am J Public Health* 1991; **81**: 1158-1162 [PMID: 1951827 DOI: 10.2105/AJPH.81.9.1158]
3 **Laing SP**, Swerdlow AJ, Slater SD, Burden AC, Morris A, Waugh NR, Gatling W, Bingley PJ, Patterson CC. Mortality from heart disease in a cohort of 23,000 patients with insulin-treated diabetes. *Diabetologia* 2003; **46**: 760-765 [PMID: 12774166 DOI: 10.1007/s00125-003-1116-6]
4 **Soedamah-Muthu SS**, Fuller JH, Mulnier HE, Raleigh VS, Lawrenson RA, Colhoun HM. High risk of cardiovascular disease in patients with type 1 diabetes in the U.K.: a cohort study using the general practice research database. *Diabetes Care* 2006; **29**: 798-804 [PMID: 16567818 DOI: 10.2337/diacare.29.04.06.dc05-1433]
5 **Soedamah-Muthu SS**, Chaturvedi N, Toeller M, Ferriss B, Reboldi P, Michel G, Manes C, Fuller JH. Risk factors for coronary heart disease in type 1 diabetic patients in Europe: the EURODIAB Prospective Complications Study. *Diabetes Care* 2004; **27**: 530-537 [PMID: 14747240 DOI: 10.2337/diacare.27.2.530]
6 **Sundquist K**, Li X. Type 1 diabetes as a risk factor for stroke

in men and women aged 15-49: a nationwide study from Sweden. *Diabet Med* 2006; **23**: 1261-1267 [PMID: 17054606 DOI: 10.1111/j.1464-5491.2006.01959.x]
7 **Grauslund J**, Jørgensen TM, Nybo M, Green A, Rasmussen LM, Sjølie AK. Risk factors for mortality and ischemic heart disease in patients with long-term type 1 diabetes. *J Diabetes Complications* 2010; **24**: 223-228 [PMID: 19577486 DOI: 10.1016/j.jdiacomp.2009.05.003]
8 **Nathan DM**, Cleary PA, Backlund JY, Genuth SM, Lachin JM, Orchard TJ, Raskin P, Zinman B. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. *N Engl J Med* 2005; **353**: 2643-2653 [PMID: 16371630 DOI: 10.1056/NEJMoa052187]
9 **Nathan DM**, Lachin J, Cleary P, Orchard T, Brillon DJ, Backlund JY, O'Leary DH, Genuth S. Intensive diabetes therapy and carotid intima-media thickness in type 1 diabetes mellitus. *N Engl J Med* 2003; **348**: 2294-2303 [PMID: 12788993 DOI: 10.1056/NEJMoa022314]
10 **Bain SC**, Gill GV, Dyer PH, Jones AF, Murphy M, Jones KE, Smyth C, Barnett AH. Characteristics of Type 1 diabetes of over 50 years duration (the Golden Years Cohort). *Diabet Med* 2003; **20**: 808-811 [PMID: 14510860 DOI: 10.1046/j.1464-5491.2003.01029.x]
11 **Larsen J**, Brekke M, Sandvik L, Arnesen H, Hanssen KF, Dahl-Jorgensen K. Silent coronary atheromatosis in type 1 diabetic patients and its relation to long-term glycemic control. *Diabetes* 2002; **51**: 2637-2641 [PMID: 12145181 DOI: 10.2337/diabetes.51.8.2637]
12 **Mäkimattila S**, Ylitalo K, Schlenzka A, Taskinen MR, Summanen P, Syvänne M, Yki-Järvinen H. Family histories of Type II diabetes and hypertension predict intima-media thickness in patients with Type I diabetes. *Diabetologia* 2002; **45**: 711-718 [PMID: 12107752 DOI: 10.1007/s00125-002-0817-6]
13 **Distiller LA**, Joffe BI, Melville V, Welman T, Distiller GB. Carotid artery intima-media complex thickening in patients with relatively long-surviving type 1 diabetes mellitus. *J Diabetes Complications* 2006; **20**: 280-284 [PMID: 16949514 DOI: 10.1016/j.jdiacomp.2005.07.012]
14 **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]
15 **Marcovecchio ML**, Dalton RN, Prevost AT, Acerini CL, Barrett TG, Cooper JD, Edge J, Neil A, Shield J, Widmer B, Todd JA, Dunger DB. Prevalence of abnormal lipid profiles and the relationship with the development of microalbuminuria in adolescents with type 1 diabetes. *Diabetes Care* 2009; **32**: 658-663 [PMID: 19171721 DOI: 10.2337/dc08-1641]
16 **Guy J**, Ogden L, Wadwa RP, Hamman RF, Mayer-Davis EJ, Liese AD, D'Agostino R, Marcovina S, Dabelea D. Lipid and lipoprotein profiles in youth with and without type 1 diabetes: the SEARCH for Diabetes in Youth case-control study. *Diabetes Care* 2009; **32**: 416-420 [PMID: 19092167 DOI: 10.2337/dc08-1775]
17 **Nikkilä EA**, Huttunen JK, Ehnholm C. Postheparin plasma lipoprotein lipase and hepatic lipase in diabetes mellitus. Relationship to plasma triglyceride metabolism. *Diabetes* 1977; **26**: 11-21 [PMID: 187516 DOI: 10.2337/diab.26.1.11]
18 **Taskinen MR**. Quantitative and qualitative lipoprotein abnormalities in diabetes mellitus. *Diabetes* 1992; **41** Suppl 2: 12-17 [PMID: 1526330 DOI: 10.2337/diab.41.2.S12]
19 **Dabelea D**, Talton JW, D'Agostino R, Wadwa RP, Urbina EM, Dolan LM, Daniels SR, Marcovina SM, Hamman RF. Cardiovascular risk factors are associated with increased arterial stiffness in youth with type 1 diabetes: the SEARCH CVD study. *Diabetes Care* 2013; **36**: 3938-3943 [PMID: 24101697 DOI: 10.2337/dc13-0851]
20 **Soedamah-Muthu SS**, Chaturvedi N, Witte DR, Stevens LK, Porta M, Fuller JH. Relationship between risk factors and mortality in type 1 diabetic patients in Europe: the EURODIAB Prospective Complications Study (PCS). *Diabetes Care* 2008; **31**: 1360-1366 [PMID: 18375412 DOI: 10.2337/dc08-0107]
21 **Molitch ME**, Rupp D, Carnethon M. Higher levels of HDL cholesterol are associated with a decreased likelihood of albuminuria in patients with long-standing type 1 diabetes. *Diabetes Care* 2006; **29**: 78-82 [PMID: 16373900 DOI: 10.2337/diacare.29.01.06.dc05-1583]
22 **Hägg S**, Thorn LM, Putaala J, Liebkind R, Harjutsalo V, Forsblom CM, Gordin D, Tatlisumak T, Groop P-H; on behalf of the FinnDiane Study Group. Incidence of Stroke According to Presence of Diabetic Nephropathy and Severe Diabetic Retinopathy in Patients With Type 1 Diabetes. *American Diabetes Association* 2013; **36**: 4140-4146 [DOI: 10.2337/dc13-0669]
23 **Writing Team for the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group**. Sustained effect of intensive treatment of type 1 diabetes mellitus on development and progression of diabetic nephropathy: the Epidemiology of Diabetes Interventions and Complications (EDIC) study. *JAMA* 2003; **290**: 2159-2167 [PMID: 14570951 DOI: 10.1001/jama.290.16.2159]
24 **de Boer IH**, Sun W, Cleary PA, Lachin JM, Molitch ME, Steffes MW, Zinman B. Intensive diabetes therapy and glomerular filtration rate in type 1 diabetes. *N Engl J Med* 2011; **365**: 2366-2376 [PMID: 22077236 DOI: 10.1056/NEJMoa1111732]
25 **Rossing P**, Hougaard P, Parving HH. Progression of microalbuminuria in type 1 diabetes: ten-year prospective observational study. *Kidney Int* 2005; **68**: 1446-1450 [PMID: 16164620 DOI: 10.1111/j.1523-1755.2005.00556.x]
26 **Rossing P**. Prediction, progression and prevention of diabetic nephropathy. The Minkowski Lecture 2005. *Diabetologia* 2006; **49**: 11-19 [PMID: 16341685 DOI: 10.1007/s00125-005-0077-3]
27 **Liew G**, Wong TY, Mitchell P, Cheung N, Wang JJ. Retinopathy predicts coronary heart disease mortality. *Heart* 2009; **95**: 391-394 [PMID: 18697802 DOI: 10.1136/hrt.2008.146670]
28 **Studies to Treat or Prevent Pediatric Type 2 Diabetes Prevention Study Group**. Prevalence of the metabolic syndrome among a racially/ethnically diverse group of U.S. eighth-grade adolescents and associations with fasting insulin and homeostasis model assessment of insulin resistance levels. *Diabetes Care* 2008; **31**: 2020-2025 [PMID: 18591405]
29 **Ghosh S**, Collier A, Hair M, Malik I, Elhadd T. MetS in type 1 diabetes. *Int J Diabetes Mellitus* 2010; **2**: 38-42 [DOI: 10.1016/j.ijdm.2009.10.005]
30 **Cleland SJ**, Fisher BM, Colhoun HM, Sattar N, Petrie JR. Insulin resistance in type 1 diabetes: what is 'double diabetes' and what are the risks? *Diabetologia* 2013; **56**: 1462-1470 [PMID: 23613085 DOI: 10.1007/s00125-013-2904-2]
31 **Williams KV**, Erbey JR, Becker D, Arslanian S, Orchard TJ. Can clinical factors estimate insulin resistance in type 1 diabetes? *Diabetes* 2000; **49**: 626-632 [PMID: 10871201 DOI: 10.2337/diabetes.49.4.626]
32 **Distiller LA**, Joffe BI, Brown V, Distiller GB. The effect of features of the metabolic syndrome on atherosclerotic risk in relatively long-surviving patients with type 1 diabetes. *Metab Syndr Relat Disord* 2010; **8**: 539-543 [PMID: 20818972 DOI: 10.1089/met.2010.0036]

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TOPIC HIGHLIGHT

Kateřina Kaňková, MD, PhD, *Series Editor*

Evidence for altered thiamine metabolism in diabetes: Is there a potential to oppose gluco- and lipotoxicity by rational supplementation?

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Abstract

Growing prevalence of diabetes (type 2 as well as type 1) and its related morbidity due to vascular complications creates a large burden on medical care worldwide. Understanding the molecular pathogenesis of chronic micro-, macro- and avascular complications mediated by hyperglycemia is of crucial importance since novel therapeutic targets can be identified and tested. Thiamine (vitamin B1) is an essential cofactor of several enzymes involved in carbohydrate metabolism and published data suggest that thiamine metabolism in diabetes is deficient. This review aims to point out the physiological role of thiamine in metabolism of glucose and amino acids, to present overview of thiamine metabolism and to describe the consequences of thiamine deficiency (either clinically manifest or latent). Furthermore, we want to explain why thiamine demands are increased in diabetes and to summarise data indicating thiamine mishandling in diabetics (by review of the studies mapping the prevalence and the degree of thiamine deficiency in diabetics). Finally, we would like to summarise the evidence for the beneficial effect of thiamine supplementation in progression of hyperglycemia-related pathology and, therefore, to justify its importance in determining the harmful impact of hyperglycemia in diabetes. Based on the data presented it could be concluded that although experimental studies mostly resulted in beneficial effects, clinical studies of appropriate size and duration focusing on the effect of thiamine supplementation/therapy on hard endpoints are missing at present. Moreover, it is not currently clear which mechanisms contribute to the deficient action of thiamine in diabetes most. Experimental studies on the molecular mechanisms of thiamine deficiency in diabetes are critically needed before clear answer to diabetes community could be given.

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Key words: Diabetes; Thiamine; Vitamin B1; Transketolase; Benfotiamine; Hyperglycemia; Nephropathy; Metabolic syndrome; Cardiovascular disease; Chronic kidney disease

Core tip: Published data suggest deficient action of thiamine in diabetes, however, it is not currently clear by which mechanisms. Plasma levels might be decreased in diabetics (although renal function has a prevailing effect), nevertheless, intracellular concentration of thiamine diphosphate is the crucial parameter and there is not a direct relationship with the plasma thiamine since the rate of transmembrane transport (*via* thiamine transporters) and intracellular activation by thiamine pyrophosphokinase might affected by hyperglycemia at first place. Experimental studies on the molecular mechanisms of thiamine deficiency in diabetes are critically needed before clear answer to diabetes community could be given.

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INTRODUCTION

Diabetes mellitus, the most common metabolic disease resulting from insufficient insulin action (either absolute or relative), is characterized by various degree of chronic hyperglycemia and is often accompanied by specific microvascular complications including nephropathy, retinopathy and neuropathy. Diabetes also substantially increases the risk of macrovascular complications (coronary heart disease, stroke and peripheral vascular disease). Both micro- and macrovascular complications affecting diabetic patients are associated with reduced quality of life and contribute substantially to considerable morbidity and mortality.

Hyperglycemia (the cumulative exposure to excess of glucose as well as individual pattern of glucose fluctuation) together with increased availability of free fatty acids (a consequence of deregulated lipolysis in adipose tissue as well as their "spill over" in case of adipocyte saturation in obese subjects) are the two dominant metabolic alterations characterising gluco- and lipotoxicity in diabetes and are causally responsible for the development of vascular complications.

Although selected aspects of thiamine metabolism abnormalities in relation to diabetes has been reviewed earlier[1,2], comprehensive view and findings from recent studies were not included. In this review we therefore aim (A) to point out the physiological role of thiamine in metabolism of glucose and amino acids, to present overview of thiamine metabolism and to describe the consequences of thiamine deficiency (either clinically manifest or latent). Furthermore, (B) we want to explain why thiamine demands are increased in diabetes and to summarise data indicating thiamine mishandling in diabetics (review of the studies mapping the prevalence and the degree of thiamine deficiency in diabetics). Finally, (C) we would like to summarise the evidence for the beneficial effect of thiamine supplementation in progression of hyperglycemia-related pathology and, therefore, to justify its importance in determining the harmful impact of hyperglycemia in diabetes.

PHYSIOLOGICAL ROLE OF THIAMINE IN GLUCOSE METABOLISM, THIAMINE METABOLISM AND CONSEQUENCES OF ITS DEFICIENCY

Role of thiamine in energy metabolism

Thiamine (vitamin B1) is a water soluble vitamin that belongs to the large group of B vitamins. Several forms of thiamine exist: (1) free thiamine; (2) thiamine monophosphate (TMP); (3) thiamine diphosphate (TDP); (4) thiamine triphosphate; and (5) adenosine thiamine triphosphate. The active form of thiamine-TDP-together with magnesium is an essential cofactor of several enzymes important for carbohydrate [transketolase (TKT), pyruvate dehydrogenase and α-ketoglutarate dehydrogenase] and amino acid (branched-chain α-keto acid dehydrogenase) metabolism[3].

Overview of thiamine metabolism

As thiamine is an essential micronutrient for humans its needs are supplied from diet rich in thiamine, such as yeast, pork, legume and cereal grains. Enzyme called thiaminase I (EC2.5.1.2), present in raw fish, shellfish, tea and coffee, decreases thiamine absorption. Thiamine is absorbed in the small intestine, predominantly in the duodenum. Thiamine esters are hydrolysed by pancreatic nucleotide pyrophosphatase (EC3.6.1.9) or alkaline phosphatase (EC3.1.3.1) to form unphosphorylated thiamine that is taken-up by enterocytes *via* thiamine transporters at low concentrations or *via* passive diffusion at higher concentrations[4]. Within enterocyte thiamine is phosphorylated by thiamine pyrophosphokinase (TPK1, EC2.7.6.2) to TDP preventing its return back to the intestinal lumen. Most of the TDP must be hydrolysed to cross the basolateral membrane using specific ATP-dependent transporter or reduced folate carrier 1 (RFC-1)[5]. Thiamine and TMP are the most abundant forms in plasma. Uptake of thiamine and TMP by cells is mediated by specific thiamine transporters 1 (THTR1 encoded by *SLC19A2* gene) and 2 (THTR2 encoded by *SLC19A3*) and RFC-1. Majority of thiamine in the cytoplasm (approximately 90%) is phosphorylated by TPK1 to TDP and used as a cofactor of cytosolic enzymes while the rest remains unphosphorylated[3]. Most of the TDP (approximately 90%) is transported into mitochondria *via* thiamine transporter from the solute carrier family of proteins encoded by the *SLC25A19* gene[6]. Two mutations in the SLC25A19 cause Amish lethal microcephaly, an autosomal recessive disorder characterized by severe microcephaly, delayed brain development, α-ketoglutaric aciduria and premature death[7]. Overview of intracellular thiamine metabolism is presented in Figure 1. Thiamine also crosses blood-brain barrier[8] and placenta[9].

Thiamine is excreted by kidneys and its rate depends on glomerular filtration, tubular reabsorption and also on plasma thiamine concentration[10]. Normally, thiamine filtered in glomerulus is effectively reabsorbed in the proximal tubule through thiamine/H^+ antiport[11]. Long-term diuretic therapy is known to produce thiamine deficiency[10]. As thiamine deficiency develops, thiamine urinary excretion falls rapidly[12].

Thiamine deficiency

Thiamine reserves are low, limited amount (up to 30 mg) is stored in skeletal muscle, brain, heart and kidneys. Thiamine stores may become depleted within weeks of

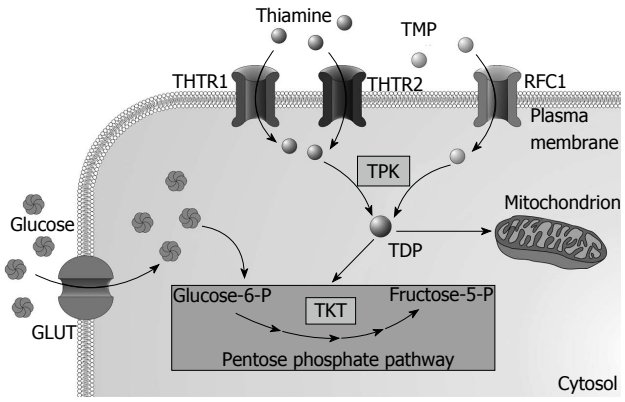

Figure 1 The overview of intracellular thiamine metabolism. GLUT: Glucose transporter; THTR1: Thiamine transporter 1; THTR2: Thiamine transporter 2; TKT: Transketolase; TPK: Thiamine pyrophosphokinase; RFC1: Reduced folate carrier 1; TDP: Thiamine diphosphate.

a deficient diet since the biological half-life of thiamine is 9 to 18 d[13]. Thiamine deficiency can result from decreased intake (most often due to its low content in diet or compromised absorption), increased demands (*e.g.,* in pregnancy) or increased renal loss. In developed countries overt thiamine deficiency due to a malnutrition is rare, however, occurs in various health conditions with alcohol abuse and chronic diseases (*e.g.,* cancer) being the most common causes. Secondary thiamine deficiency can also accompany heart failure, severe infections or long-term diuretic use.

Although all cell types utilize thiamine, the nervous system is particularly sensitive to thiamine deficiency due to its role in the synthesis of acetylcholine and γ-aminobutyric acid in the brain. Also the heart is strongly sensitive to thiamine limitation due to the high level of oxidative metabolism. Early symptoms of thiamine deficiency are in general nonspecific including fatigue, anorexia, nausea, weight loss and depression. Serious thiamine deficiency can clinically manifest as beriberi, Wernicke's encephalopathy or Korsakoff's psychosis[14]. Beriberi, classically categorized as dry or wet, is present in populations relying on diet constituting predominantly of polished rice (very low thiamine content). Wet beriberi (also known as thiamine deficiency with cardiopathy) affects primarily heart and can lead to a congestive heart failure with peripheral oedemas, tachycardia, dyspnoea and weakness[15]. Patients with dry form usually suffer from peripheral neuropathy leading to paralysis, weakness, leg paraesthesia, wasting of muscle and various other symptoms.

Thiamine deficiency is common in alcoholics as alcohol negatively affects thiamine uptake and intracellular phosphorylation, thus contributing to a marked thiamine deficiency. Central nervous system manifestations of thiamine deficiency in alcoholics are known as Wernicke-Korsakoff syndrome. The symptoms include changes of mental status (*e.g.,* confusion), ocular signs (nystagmus) and ataxia. Thiamine deficiency in alcoholics can also be accompanied by severe loss of memory denoted as Korsakoff psychosis. Both symptoms commonly occurs together constituting so called Wernicke-Korsakoff syndrome[16].

Intracellular thiamine deficit due to mutations in the gene *SLC19A2* encoding for THTR1 causes thiamine-responsive megaloblastic anaemia syndrome (TRMA)[17]. TRMA is an autosomal recessive disorder that typically manifests as megaloblastic anaemia, hearing loss and diabetes[18].

Supplementation in case of proven thiamine deficiency can be achieved by free thiamine that was shown to increase plasma thiamine levels as well as intracellular TDP although the rate of thiamine transport through the plasma membrane is quite slow[19]. Several lipophilic thiamine derivatives have been synthesized (*e.g.,* fursultiamine and sulbutiamine) which are able to diffuse through plasma membrane independent of transporters thus being more effective than free thiamine. Within the cell they are converted to thiamine. Benfotiamine (S-benzoylthiamine O-monophosphate) is another derivative with better availability than thiamine (reflected by higher plasma thiamine levels). However benfotiamine must be dephosphorylated to S-benzoylthiamine by ecto-alkaline phosphatase to become lipophilic prior crossing plasma membrane. No adverse effects of either high-dose thiamine or benfotiamine supplementation have been reported so far probably due to an efficient renal excretion or rapid uptake by hepatocytes with subsequent transformation to thiamine and release into the blood, respectively[19].

Laboratory test used for estimation of thiamine status

The two main tests routinely used for the assessment of thiamine status are the measurement of erythrocyte TKT activity and the so called thiamine effect. The former is measured by a kinetic reaction without adding thiamine. Thiamine effect expresses the increase of TKT activity after addition of saturating amount of thiamine to the reaction. The increase up to 15% is considered as normal thiamine status, higher increase is an indicator of mild (up to 25%) or severe (more than 25%) thiamine deficiency[15]. Plasma thiamine levels can also be measured although they predominantly reflect thiamine intake rather than cellular levels. Combination of erythrocyte TKT activity and thiamine effect measurement is considered as the most reliable indicator of thiamine status in clinical settings.

DIABETES AS A STATE OF INCREASED DEMAND FOR THIAMINE AND THE EVIDENCE FOR THE ALTERED THIAMINE METABOLISM IN DIABETES

Consequences of hyperglycemia for thiamine availability

Diabetes of all types is *ex definitione* characterised by hyperglycemia. Contribution of fasting and postprandial glucose elevation is variable though in various degrees of abnormal glucose tolerance and most likely also interindividually. Increased glucose supply stimulates its intracellular metabolism (glycolysis) with subsequent increase in

the production of reactive oxygen species (ROS) in mitochondria[20,21]. Overproduction of ROS in mitochondria links- *via* inhibition of the key glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase-hyperglycemia with activation of several biochemical pathways involved in the development of microvascular complications of diabetes incl. hexosamine and polyol pathways, production of advanced glycation end products (AGEs) and activation of protein kinase C[22]. However, cells in general are capable of either decreasing overproduction of ROS by enzymatic and non-enzymatic antioxidant mechanisms and/or eliminating of damaging metabolites and their substrates (generated by overloaded glycolysis) that accumulate within cells. Pentose phosphate pathway (PPP) is an example of the latter mechanism. PPP represents an alternative pathway for glucose oxidation fulfilling three important functions: (1) production of reducing equivalent NADPH necessary for reduction of oxidized glutathione thus supporting intracellular antioxidant defence; (2) production of ribose-5-phosphate required for the synthesis of nucleotides; and (3) metabolic use of pentoses obtained from the diet. PPP consists of two branches: (1) irreversible oxidative branch necessary for NADPH and pentose phosphates production; and (2) reversible non-oxidative branch in which interconversion of three to seven carbons containing sugars occurs. TKT (EC 2.2.1.1), one of the key enzymes of non-oxidative branch of PPP, can limit the activation of damaging pathways through lowering availability of their precursors. TKT transports two-carbon units and catalyses formation of ribose-5-phosphate from glycolytic intermediates. As a cofactor of TKT, thiamine may have a profound effect on glucose metabolism through the regulation of PPP and indeed, TKT activation by benfotiamine (see below) in endothelial cells blocked several pathways responsible for hyperglycemic damage and prevented development and progression of diabetic complications in animal models[23]. The mechanism responsible for the observed effect upon activation of non-oxidative reversible branch of PPP by thiamine or its derivative benfotiamine was the diminished accumulation of triosephosphates and fructose-6-phosphate induced by hyperglycemia[2].

Thiamine mishandling in diabetes

Little is known about the precise mechanisms how diabetes affects thiamine metabolism. Patients with type 1 and 2 diabetes mellitus (T1DM and T2DM) do not have a marked thiamine deficiency [conventionally defined as an increase of TKT activity in red blood cells (RBC) higher than 15% after addition of saturating amount of TDP]. However, plasma thiamine levels in diabetics are decreased by 75% compared to healthy subjects[24]. RFC-1 and THTR1 protein expression in RBCs obtained from diabetic patients (both T1DM and T2DM) is higher than in healthy subjects[24].

Experimental evidence suggests abnormal thiamine handling in the kidneys in diabetes that might be one of the reasons for decreased plasma thiamine levels in diabetics. Incubation of human primary proximal tubule cells in high glucose conditions (26 mmol/L) decreases both mRNA and protein expression of THTR1 and THTR2 compared to 5 mmol/L glucose[25]. Renal clearance of thiamine is increased by 8-fold in experimental model of diabetes. Interestingly, increased clearance was prevented by high-dose thiamine supplementation[26]. Thiamine renal clearance is also increased in subjects with T1DM (by 24-fold) and T2DM (by 16-fold)[24].

Further changes of thiamine metabolism probably occur with the development of chronic diabetic microvascular complications namely diabetic nephropathy together with chronic kidney disease (CKD). While in diabetics with preserved renal function plasma thiamine levels tend to be lower most likely on account of increased renal clearance, in subjects with CKD stages corresponding with renal insufficiency and failure the situation dramatically changes. We have previously comprehensively studied plasma and intracellular parameters of thiamine metabolism in diabetics with the aim to dissect the complex relationships between the effect of diabetes and renal function[27]. We reported that plasma levels of thiamine and its esters and TKT activity in RBCs increased with severity of diabetic nephropathy (and CKD respectively) being highest in subjects with end-stage renal disease, however, levels of TDP in RBCs did not show proportional trend. Since the effectiveness of intracellular TDP production depends on substrate availability (*i.e.,* the rate of transmembrane transport *via* thiamine transporters) and TPK activity we therefore hypothesized that these could be the processes diminished by hyperglycemia and the causal reasons for the failure of protective action of PPP under hyperglycemia. While T1DM and T2DM patients with normal renal function have been shown to have a higher expression of THTR1 and THTR2 in mononuclear cells compared to healthy subjects by one study[28], data on TPK activity and THTR2 expression in diabetes are missing at all. Obviously, there is still a large gap in our understanding of the precise molecular mechanisms of thiamine deficiency and the problem definitely warrants further study.

OVERVIEW OF *IN VITRO*, ANIMAL AND HUMAN STUDIES WITH THIAMINE OR BENFOTIAMINE SUPPLEMENTATION IN DIABETIC CONDITIONS

In vitro studies

Several studies explored the effect of thiamine and/or benfotiamine on pathways implicated in the pathogenesis of hyperglycemia-induced damage *in vitro*. Cultivation of RBC in hyperglycemia with addition of thiamine increased activity of TKT, decreased production of triose phosphates and methylglyoxal and increased concentrations of sedoheptulose-7-phosphate and ribose-5-phosphate[29]. Benfotiamine as well as thiamine have been shown to correct defective replication of human umbilical vein endothelial cells (HUVEC) and to decrease their

production of AGEs induced by hyperglycemia[30]. Thiamine also suppressed markers of endothelial cell damage (inhibited cell migration and increased von Willebrand factor secretion) induced by hyperglycemia in bovine aortic endothelial cells[31]. Both thiamine and benfotiamine decreased activation of polyol pathway (aldose reductase mRNA expression, enzyme activity and intracellular levels of sorbitol) while increasing expression and activity of TKT in HUVEC and bovine retinal pericytes cultured in hyperglycemia[32]. Notably, benfotiamine restored impairment of endothelial progenitor cells differentiation caused by hyperglycemia[33]. Possible benfotiamine antioxidant properties and protective effect on DNA have also been investigated. Benfotiamine prevented oxidative stress (probably through direct antioxidant effect) and also DNA damage[34]. The same study also confirmed that benfotiamine increased TKT expression and activity. Intermittent exposure of human retinal pericytes to fluctuating glucose levels induced their apoptosis, the effect was however prevented by thiamine and benfotiamine[35]. It has also been studied whether thiamine and/or benfotiamine affect glucose and lipid metabolism in human skeletal muscle cells. Benfotiamine but not thiamine increased glucose oxidation while lipid oxidation and metabolism was influenced by neither of the two. Benfotiamine also down-regulated NADPH oxidase 4 expression[36].

Animal models

The first published study exploring the effect of thiamine and benfotiamine supplementation on peripheral nerve function and production of AGEs in diabetic rats found that benfotiamine but not thiamine had protective effect with respect to both processes[37]. Already mentioned key study provided evidence for the role of PPP in diabetes showing that benfotiamine (activating TKT) inhibited three harmful pathways and NF-κ signalling activated by hyperglycemia and prevented development of diabetic retinopathy in experimental rats[23]. The group of Thornalley published a series of papers investigating the effect of thiamine and/or benfotiamine supplementation on the development of diabetic microvascular complications, predominantly diabetic nephropathy. They found that thiamine and benfotiamine were able to suppress the accumulation of AGEs in the kidney, eye, nerves and plasma of diabetic rats[38]. Furthermore, they reported that high-dose thiamine and benfotiamine therapy prevented diabetic nephropathy through increased TKT expression, decreased level of triosephosphates a decreased protein kinase C activation. Importantly, since no changes in fasting plasma glucose and HbA1c were observed this effect is independent of diabetes compensation[26]. Furthermore, high-dose thiamine therapy had positive effect on diabetes-induced dyslipidaemia (preventing the increase of plasma cholesterol and triglycerides but not high-density lipoprotein decrease). Benfotiamine and low-dose thiamine failed to achieve the same effect[39]. They also quantified AGEs in plasma of diabetic rats. Both thiamine and benfotiamine supplementation have been shown to normalize AGEs derived from methylglyoxal and glyoxal. On the contrary, carboxy methyl lysine and N-epsilon(1-carboxyethyl)lysine residues have been normalized by thiamine only[40]. Finally, they quantified protein damage caused by glycation, oxidation and nitration in diabetic rats and found increased AGEs content in the diabetic kidney, eye, nerve and plasma that was reversed by thiamine and benfotiamine therapy. Thiamine itself also reversed increase of plasma glycation free adducts. Both therapies reversed increased urinary excretion of glycation, oxidation and nitration free adducts[41]. Several studies evaluated the effect of thiamine/benfotiamine treatment with respect to heart function in diabetes animal model. Benfotiamine alleviated abnormalities in parameters related to the contractile dysfunction in diabetic mouse. It also reduced oxidative stress induced by diabetes however production of AGEs was unchanged[42]. High-dose thiamine therapy prevented diabetes-induced cardiac fibrosis through increased expression of genes with pro-fibrotic effect and decreased matrix metalloproteinase activity in hearts of diabetic rats[43]. Another study revealed that benfotiamine therapy protected diabetic mice from heart failure with several pathogenic mechanism suggested including improved cardiac perfusion, reduced fibrosis and cardiomyocyte apoptosis[44]. Same authors found that benfotiamine improved prognosis of diabetic mice after myocardial infarction in terms of survival, functional recovery, reduced cardiomyocyte apoptosis and neurohormonal activation[45]. The same was true for control non-diabetic mice probably due to increased activity of pyruvate dehydrogenase in hearts of diabetic rats by thiamine treatment. Subsequent *in vitro* experiment revealed that responsible molecular mechanism may be suppression of O-glycosylated protein[46]. Both *in vitro* and *in vivo* benfotiamine supplementation had positive effect on cardiac progenitor cells in terms of their proliferation, abundance, functionality and TKT activity (all listed parameters being compromised by hyperglycemia)[47]. In mouse diabetes model of limb ischemia benfotiamine increased TKT activity, prevented toe necrosis, improved perfusion and restored vasodilation. Moreover, benfotiamine prevented accumulation of AGEs in vessels and inhibited pro-apoptotic caspase-3 in muscles[48]. Another work assessed cerebral oxidative stress in diabetic mice. Benfotiamine was found to lower oxidative stress (estimated as reduced/oxidized glutathione) however levels of AGEs, protein carbonyl and tumor necrosis factor-α were unchanged[49]. Administration of benfotiamine and fenofibrate alone or in combination attenuated endothelial dysfunction and nephropathy in diabetic rats. Lipid profile however was normalized only by fenofibrate not by benfotiamine[50].

Human studies

Only few studies in diabetic patients have been published so far that explored the effect of thiamine or benfotiamine treatment on hard endpoints, *i.e.,* development or progression of clinically manifest diabetic complications, namely kidney disease and neuropathy. In the pilot study,

Table 1 The effect of thiamine or benfotiamine supplementation on surrogate markers related to hyperglycemia in human studies

Ref.	Treatment	Results
Arora *et al*[56]	Thiamine	Improved endothelial-dependent vasodilation in subjects with impaired glucose tolerance and in T2DM patients
Du *et al*[57]	Benfotiamine + α-lipoic acid	Normalization of AGEs production and prostacyclin synthase activity and decreases hexosamine-modified protein in monocytes without changing glycaemic control in T1DM
González-Ortiz *et al*[58]	Thiamine	Lower blood glucose and leptin in T2DM patients
Polizzi *et al*[59]	Thiamine + vitamin B6	Administration of vitamin B6 together with thiamine but not B6 itself decreases DNA glycation in T2DM
Riaz *et al*[60]	Thiamine	Decreased albumin in urine in T2DM patients
Schupp *et al*[61]	Benfotiamine	Decreased genomic damage in peripheral lymphocytes in haemodialysis patients
Stirban *et al*[62]	Benfotiamine	No effect on skin autofluorescence in T2DM patients
Stirban *et al*[63]	Benfotiamine	No effect on flow-mediated dilation in T2DM patients

T1DM: Type 1 diabetes mellitus; AGEs: Advanced glycation end products; T2DM: Type 2 diabetes mellitus.

high-dose thiamine therapy for 3 mo significantly decreased urinary albumin excretion (UAE) without affecting glycaemic control, lipids and blood pressure in T2DM patients[51]. In another study however, 3 mo of benfotiamine therapy improved thiamine status (assessed as a TKT activity and the whole blood thiamine concentration) but did not change UAE and/or kidney marker of tubular damage in T2DM patients[52]. The same authors also determined AGEs production and markers of endothelial dysfunction and low-grade inflammation in the same cohort. Benfotiamine did not affect any of the ascertained markers[53]. In patients with diabetic neuropathy, short-term benfotiamine therapy was found to improve neuropathy score and to decrease the pain perception[54]. In the recent study, long-term (1 year) benfotiamine therapy did not affect peripheral nerve function and soluble inflammatory markers (*e.g.*, interleukin-6 or E-selectin) despite significantly increasing the whole blood levels of thiamine and TDP in T1DM patients[55]. This study was however criticized for inappropriate study design and definition of end-points[55]. Several other studies in human diabetics explored various surrogate markers related to pathologic processes occurring in hyperglycemia, the results are summarized in Table 1.

CONCLUSION

Since glucose metabolism depends on thiamine as an enzyme cofactor, it is biologically feasible to suppose that adequate thiamine supplementation in diabetics might have a profound effect on metabolic compensation and thus development of vascular complications. It could also possibly influence earlier stages of abnormal glucose tolerance such as components of metabolic syndrome. Data on surrogate markers of endothelial dysfunction and cardiovascular disease indicate that thiamine could be of interest also for the broader spectrum of diseases apart from diabetes. While experimental studies mostly resulted in beneficial effects clinical studies of appropriate size and duration focusing on the effect of thiamine supplementation/therapy on hard endpoints are missing at present. Moreover, it is not currently clear which mechanisms contribute to the deficient action of thiamine most. Based on the data presented boosting solely plasma levels might not be the right way to go since intracellular TDP levels are not a mere reflection of the plasma levels of their precursor. Apparently experimental studies on the molecular mechanisms of thiamine deficiency in diabetes are critically needed before giving clear answer to diabetes community.

REFERENCES

1 **Page GL**, Laight D, Cummings MH. Thiamine deficiency in diabetes mellitus and the impact of thiamine replacement on glucose metabolism and vascular disease. *Int J Clin Pract* 2011; **65**: 684-690 [PMID: 21564442 DOI: 10.1111/j.1742-1241.2011.02680.x]

2 **Rabbani N**, Thornalley PJ. Emerging role of thiamine therapy for prevention and treatment of early-stage diabetic nephropathy. *Diabetes Obes Metab* 2011; **13**: 577-583 [PMID: 21342411 DOI: 10.1111/j.1463-1326.2011.01384.x]

3 **Gangolf M**, Czerniecki J, Radermecker M, Detry O, Nisolle M, Jouan C, Martin D, Chantraine F, Lakaye B, Wins P, Grisar T, Bettendorff L. Thiamine status in humans and content of phosphorylated thiamine derivatives in biopsies and cultured cells. *PLoS One* 2010; **5**: e13616 [PMID: 21049048 DOI: 10.1371/journal.pone.0013616]

4 **Rindi G**, Laforenza U. Thiamine intestinal transport and related issues: recent aspects. *Proc Soc Exp Biol Med* 2000; **224**: 246-255 [PMID: 10964259]

5 **Ganapathy V**, Smith SB, Prasad PD. SLC19: the folate/thiamine transporter family. *Pflugers Arch* 2004; **447**: 641-646 [PMID: 14770311 DOI: 10.1007/s00424-003-1068-1]

6 **Bettendorff L**. The compartmentation of phosphorylated thiamine derivatives in cultured neuroblastoma cells. *Biochim Biophys Acta* 1994; **1222**: 7-14 [PMID: 8186267]

7 **Lindhurst MJ**, Fiermonte G, Song S, Struys E, De Leonardis F, Schwartzberg PL, Chen A, Castegna A, Verhoeven N, Mathews CK, Palmieri F, Biesecker LG. Knockout of Slc25a19 causes mitochondrial thiamine pyrophosphate depletion, embryonic lethality, CNS malformations, and anemia. *Proc Natl Acad Sci USA* 2006; **103**: 15927-15932 [PMID: 17035501 DOI: 10.1073/pnas.0607661103]

8 **Patrini C**, Reggiani C, Laforenza U, Rindi G. Blood-brain transport of thiamine monophosphate in the rat: a kinetic study in vivo. *J Neurochem* 1988; **50**: 90-93 [PMID: 3335853]

9 **Rajgopal A**, Edmondnson A, Goldman ID, Zhao R. SLC19A3 encodes a second thiamine transporter ThTr2. *Biochim Biophys Acta* 2001; **1537**: 175-178 [PMID: 11731220]

10 **Sica DA**. Loop diuretic therapy, thiamine balance, and heart failure. *Congest Heart Fail* 2007; **13**: 244-247 [PMID: 17673878

DOI: 10.1111/j.1527-5299.2007.06260.x]
11 **Gastaldi G**, Cova E, Verri A, Laforenza U, Faelli A, Rindi G. Transport of thiamin in rat renal brush border membrane vesicles. *Kidney Int* 2000; **57**: 2043-2054 [PMID: 10792623 DOI: 10.1046/j.1523-1755.2000.00053.x]
12 **Wooley JA**. Characteristics of thiamin and its relevance to the management of heart failure. *Nutr Clin Pract* 2008; **23**: 487-493 [PMID: 18849553 DOI: 10.1177/0884533608323430]
13 **Rucker RBZJ**, Suttie JW, McCormick DB. Handbook of vitamins. CRC Press, 2007
14 **McCandless D**. Thiamine deficiency and associated clinical disorders. Humana Press, 2009
15 **Brady JA**, Rock CL, Horneffer MR. Thiamin status, diuretic medications, and the management of congestive heart failure. *J Am Diet Assoc* 1995; **95**: 541-544 [PMID: 7722187 DOI: 10.1016/S0002-8223(95)00148-4]
16 **Kopelman MD**, Thomson AD, Guerrini I, Marshall EJ. The Korsakoff syndrome: clinical aspects, psychology and treatment. *Alcohol Alcohol* 2009; **44**: 148-154 [PMID: 19151162 DOI: 10.1093/alcalc/agn118]
17 **Diaz GA**, Banikazemi M, Oishi K, Desnick RJ, Gelb BD. Mutations in a new gene encoding a thiamine transporter cause thiamine-responsive megaloblastic anaemia syndrome. *Nat Genet* 1999; **22**: 309-312 [PMID: 10391223 DOI: 10.1038/10385]
18 **Neufeld EJ**, Fleming JC, Tartaglini E, Steinkamp MP. Thiamine-responsive megaloblastic anemia syndrome: a disorder of high-affinity thiamine transport. *Blood Cells Mol Dis* 2010; **27**: 135-138 [PMID: 11358373 DOI: 10.1006/bcmd.2000.0356]
19 **Volvert ML**, Seyen S, Piette M, Evrard B, Gangolf M, Plumier JC, Bettendorff L. Benfotiamine, a synthetic S-acyl thiamine derivative, has different mechanisms of action and a different pharmacological profile than lipid-soluble thiamine disulfide derivatives. *BMC Pharmacol* 2008; **8**: 10 [PMID: 18549472 DOI: 10.1186/1471-2210-8-10]
20 **Mokini Z**, Marcovecchio ML, Chiarelli F. Molecular pathology of oxidative stress in diabetic angiopathy: role of mitochondrial and cellular pathways. *Diabetes Res Clin Pract* 2010; **87**: 313-321 [PMID: 20022399 DOI: 10.1016/j.diabres.2009.11.018]
21 **Naudi A**, Jove M, Ayala V, Cassanye A, Serrano J, Gonzalo H, Boada J, Prat J, Portero-Otin M, Pamplona R. Cellular dysfunction in diabetes as maladaptive response to mitochondrial oxidative stress. *Exp Diabetes Res* 2012; **2012**: 696215 [PMID: 22253615 DOI: 10.1155/2012/696215]
22 **Brownlee M**. Biochemistry and molecular cell biology of diabetic complications. *Nature* 2001; **414**: 813-820 [PMID: 11742414 DOI: 10.1038/414813a]
23 **Hammes HP**, Du X, Edelstein D, Taguchi T, Matsumura T, Ju Q, Lin J, Bierhaus A, Nawroth P, Hannak D, Neumaier M, Bergfeld R, Giardino I, Brownlee M. Benfotiamine blocks three major pathways of hyperglycemic damage and prevents experimental diabetic retinopathy. *Nat Med* 2003; **9**: 294-299 [PMID: 12592403 DOI: 10.1038/nm834]
24 **Thornalley PJ**, Babaei-Jadidi R, Al Ali H, Rabbani N, Antonysunil A, Larkin J, Ahmed A, Rayman G, Bodmer CW. High prevalence of low plasma thiamine concentration in diabetes linked to a marker of vascular disease. *Diabetologia* 2007; **50**: 2164-2170 [PMID: 17676306]
25 **Larkin JR**, Zhang F, Godfrey L, Molostvov G, Zehnder D, Rabbani N, Thornalley PJ. Glucose-induced down regulation of thiamine transporters in the kidney proximal tubular epithelium produces thiamine insufficiency in diabetes. *PLoS One* 2012; **7**: e53175 [PMID: 23285265 DOI: 10.1371/journal.pone.0053175]
26 **Babaei-Jadidi R**, Karachalias N, Ahmed N, Battah S, Thornalley PJ. Prevention of incipient diabetic nephropathy by high-dose thiamine and benfotiamine. *Diabetes* 2003; **52**: 2110-2120 [PMID: 12882930 DOI: 10.2337/diabetes.52.8.2110]
27 **Pácal L**, Tomandl J, Svojanovsky J, Krusová D, Stepánková S, Rehorová J, Olsovsky J, Belobrádková J, Tanhäuserová V, Tomandlová M, Muzík J, Kanková K. Role of thiamine status and genetic variability in transketolase and other pentose phosphate cycle enzymes in the progression of diabetic nephropathy. *Nephrol Dial Transplant* 2011; **26**: 1229-1236 [PMID: 20826743 DOI: 10.1093/ndt/gfq550]
28 **Antonysunil A**, Babaei-Jadidi R, Rabbani N, Ahmed A, Rayman G, Bodmer CW, Thornalley PJ. Increased thiamine transporter contents of red blood cells and peripheral blood mononuclear leukocytes in type 1 and type 2 diabetic patients. *Diabetes* 2007; **56**: A609
29 **Thornalley PJ**, Jahan I, Ng R. Suppression of the accumulation of triosephosphates and increased formation of methylglyoxal in human red blood cells during hyperglycaemia by thiamine in vitro. *J Biochem* 2001; **129**: 543-549 [PMID: 11275553]
30 **Pomero F**, Molinar Min A, La Selva M, Allione A, Molinatti GM, Porta M. Benfotiamine is similar to thiamine in correcting endothelial cell defects induced by high glucose. *Acta Diabetol* 2001; **38**: 135-138 [PMID: 11827434]
31 **Ascher E**, Gade PV, Hingorani A, Puthukkeril S, Kallakuri S, Scheinman M, Jacob T. Thiamine reverses hyperglycemia-induced dysfunction in cultured endothelial cells. *Surgery* 2001; **130**: 851-858 [PMID: 11685195]
32 **Berrone E**, Beltramo E, Solimine C, Ape AU, Porta M. Regulation of intracellular glucose and polyol pathway by thiamine and benfotiamine in vascular cells cultured in high glucose. *J Biol Chem* 2006; **281**: 9307-9313 [PMID: 16452468 DOI: 10.1074/jbc.M600418200]
33 **Marchetti V**, Menghini R, Rizza S, Vivanti A, Feccia T, Lauro D, Fukamizu A, Lauro R, Federici M. Benfotiamine counteracts glucose toxicity effects on endothelial progenitor cell differentiation via Akt/FoxO signaling. *Diabetes* 2006; **55**: 2231-2237 [PMID: 16873685 DOI: 10.2337/db06-0369]
34 **Schmid U**, Stopper H, Heidland A, Schupp N. Benfotiamine exhibits direct antioxidative capacity and prevents induction of DNA damage in vitro. *Diabetes Metab Res Rev* 2008; **24**: 371-377 [PMID: 18384109 DOI: 10.1002/dmrr.860]
35 **Beltramo E**, Nizheradze K, Berrone E, Tarallo S, Porta M. Thiamine and benfotiamine prevent apoptosis induced by high glucose-conditioned extracellular matrix in human retinal pericytes. *Diabetes Metab Res Rev* 2009; **25**: 647-656 [PMID: 19768736 DOI: 10.1002/dmrr.1008]
36 **Fraser DA**, Hessvik NP, Nikolić N, Aas V, Hanssen KF, Bøhn SK, Thoresen GH, Rustan AC. Benfotiamine increases glucose oxidation and downregulates NADPH oxidase 4 expression in cultured human myotubes exposed to both normal and high glucose concentrations. *Genes Nutr* 2012; **7**: 459-469 [PMID: 21984258 DOI: 10.1007/s12263-011-0252-8]
37 **Stracke H**, Hammes HP, Werkmann D, Mavrakis K, Bitsch I, Netzel M, Geyer J, Köpcke W, Sauerland C, Bretzel RG, Federlin KF. Efficacy of benfotiamine versus thiamine on function and glycation products of peripheral nerves in diabetic rats. *Exp Clin Endocrinol Diabetes* 2001; **109**: 330-336 [PMID: 11571671 DOI: 10.1055/s-2001-17399]
38 **Karachalias N**, Babaei-Jadidi R, Ahmed N, Thornalley PJ. Accumulation of fructosyl-lysine and advanced glycation end products in the kidney, retina and peripheral nerve of streptozotocin-induced diabetic rats. *Biochem Soc Trans* 2003; **31**: 1423-1425 [PMID: 14641079]
39 **Babaei-Jadidi R**, Karachalias N, Kupich C, Ahmed N, Thornalley PJ. High-dose thiamine therapy counters dyslipidaemia in streptozotocin-induced diabetic rats. *Diabetologia* 2004; **47**: 2235-2246 [PMID: 15662560 DOI: 10.1007/s00125-004-1582-5]
40 **Karachalias N**, Babaei-Jadidi R, Kupich C, Ahmed N, Thornalley PJ. High-dose thiamine therapy counters dyslipidemia and advanced glycation of plasma protein in streptozotocin-induced diabetic rats. *Ann N Y Acad Sci* 2005; **1043**: 777-783 [PMID: 16037305 DOI: 10.1196/annals.1333.090]
41 **Karachalias N**, Babaei-Jadidi R, Rabbani N, Thornalley PJ. Increased protein damage in renal glomeruli, retina, nerve,

plasma and urine and its prevention by thiamine and benfotiamine therapy in a rat model of diabetes. *Diabetologia* 2010; **53**: 1506-1516 [PMID: 20369223 DOI: 10.1007/s00125-010-1722-z]
42 **Ceylan-Isik AF**, Wu S, Li Q, Li SY, Ren J. High-dose benfotiamine rescues cardiomyocyte contractile dysfunction in streptozotocin-induced diabetes mellitus. *J Appl Physiol* (1985) 2006; **100**: 150-156 [PMID: 16166234 DOI: 10.1152/japplphysiol.00988.2005]
43 **Kohda Y**, Shirakawa H, Yamane K, Otsuka K, Kono T, Terasaki F, Tanaka T. Prevention of incipient diabetic cardiomyopathy by high-dose thiamine. *J Toxicol Sci* 2008; **33**: 459-472 [PMID: 18827445 DOI: 10.2131/jts.33.459]
44 **Katare RG**, Caporali A, Oikawa A, Meloni M, Emanueli C, Madeddu P. Vitamin B1 analog benfotiamine prevents diabetes-induced diastolic dysfunction and heart failure through Akt/Pim-1-mediated survival pathway. *Circ Heart Fail* 2010; **3**: 294-305 [PMID: 20107192 DOI: 10.1161/CIRCHEARTFAILURE.109.903450]
45 **Katare R**, Caporali A, Emanueli C, Madeddu P. Benfotiamine improves functional recovery of the infarcted heart via activation of pro-survival G6PD/Akt signaling pathway and modulation of neurohormonal response. *J Mol Cell Cardiol* 2010; **49**: 625-638 [PMID: 20542491 DOI: 10.1016/j.yjmcc.2010.05.014]
46 **Kohda Y**, Umeki M, Kono T, Terasaki F, Matsumura H, Tanaka T. Thiamine ameliorates diabetes-induced inhibition of pyruvate dehydrogenase (PDH) in rat heart mitochondria: investigating the discrepancy between PDH activity and PDH E1alpha phosphorylation in cardiac fibroblasts exposed to high glucose. *J Pharmacol Sci* 2010; **113**: 343-352 [PMID: 20644337 DOI: 10.1254/jphs.09359FP]
47 **Katare R**, Oikawa A, Cesselli D, Beltrami AP, Avolio E, Muthukrishnan D, Munasinghe PE, Angelini G, Emanueli C, Madeddu P. Boosting the pentose phosphate pathway restores cardiac progenitor cell availability in diabetes. *Cardiovasc Res* 2013; **97**: 55-65 [PMID: 22997160 DOI: 10.1093/cvr/cvs291]
48 **Gadau S**, Emanueli C, Van Linthout S, Graiani G, Todaro M, Meloni M, Campesi I, Invernici G, Spillmann F, Ward K, Madeddu P. Benfotiamine accelerates the healing of ischaemic diabetic limbs in mice through protein kinase B/Akt-mediated potentiation of angiogenesis and inhibition of apoptosis. *Diabetologia* 2006; **49**: 405-420 [PMID: 16416271 DOI: 10.1007/s00125-005-0103-5]
49 **Wu S**, Ren J. Benfotiamine alleviates diabetes-induced cerebral oxidative damage independent of advanced glycation end-product, tissue factor and TNF-alpha. *Neurosci Lett* 2006; **394**: 158-162 [PMID: 16260089 DOI: 10.1016/j.neulet.2005.10.022]
50 **Balakumar P**, Chakkarwar VA, Singh M. Ameliorative effect of combination of benfotiamine and fenofibrate in diabetes-induced vascular endothelial dysfunction and nephropathy in the rat. *Mol Cell Biochem* 2009; **320**: 149-162 [PMID: 18830571 DOI: 10.1007/s11010-008-9917-z]
51 **Rabbani N**, Alam SS, Riaz S, Larkin JR, Akhtar MW, Shafi T, Thornalley PJ. High-dose thiamine therapy for patients with type 2 diabetes and microalbuminuria: a randomised, double-blind placebo-controlled pilot study. *Diabetologia* 2009; **52**: 208-212 [PMID: 19057893 DOI: 10.1007/s00125-008-1224-4]
52 **Alkhalaf A**, Klooster A, van Oeveren W, Achenbach U, Kleefstra N, Slingerland RJ, Mijnhout GS, Bilo HJ, Gans RO, Navis GJ, Bakker SJ. A double-blind, randomized, placebo-controlled clinical trial on benfotiamine treatment in patients with diabetic nephropathy. *Diabetes Care* 2010; **33**: 1598-1601 [PMID: 20413516 DOI: 10.2337/dc09-2241]
53 **Alkhalaf A**, Kleefstra N, Groenier KH, Bilo HJ, Gans RO, Heeringa P, Scheijen JL, Schalkwijk CG, Navis GJ, Bakker SJ. Effect of benfotiamine on advanced glycation endproducts and markers of endothelial dysfunction and inflammation in diabetic nephropathy. *PLoS One* 2012; **7**: e40427 [PMID: 22792314 DOI: 10.1371/journal.pone.0040427]
54 **Haupt E**, Ledermann H, Köpcke W. Benfotiamine in the treatment of diabetic polyneuropathy--a three-week randomized, controlled pilot study (BEDIP study). *Int J Clin Pharmacol Ther* 2005; **43**: 71-77 [PMID: 15726875]
55 **Fraser DA**, Diep LM, Hovden IA, Nilsen KB, Sveen KA, Seljeflot I, Hanssen KF. The effects of long-term oral benfotiamine supplementation on peripheral nerve function and inflammatory markers in patients with type 1 diabetes: a 24-month, double-blind, randomized, placebo-controlled trial. *Diabetes Care* 2012; **35**: 1095-1097 [PMID: 22446172 DOI: 10.2337/dc11-1895]
56 **Arora S**, Lidor A, Abularrage CJ, Weiswasser JM, Nylen E, Kellicut D, Sidawy AN. Thiamine (vitamin B1) improves endothelium-dependent vasodilatation in the presence of hyperglycemia. *Ann Vasc Surg* 2006; **20**: 653-658 [PMID: 16741654]
57 **Du X**, Edelstein D, Brownlee M. Oral benfotiamine plus alpha-lipoic acid normalises complication-causing pathways in type 1 diabetes. *Diabetologia* 2008; **51**: 1930-1932 [PMID: 18663426 DOI: 10.1007/s00125-008-1100-2]
58 **González-Ortiz M**, Martínez-Abundis E, Robles-Cervantes JA, Ramírez-Ramírez V, Ramos-Zavala MG. Effect of thiamine administration on metabolic profile, cytokines and inflammatory markers in drug-naïve patients with type 2 diabetes. *Eur J Nutr* 2011; **50**: 145-149 [PMID: 20652275 DOI: 10.1007/s00394-010-0123-x]
59 **Polizzi FC**, Andican G, Çetin E, Civelek S, Yumuk V, Burçak G. Increased DNA-glycation in type 2 diabetic patients: the effect of thiamine and pyridoxine therapy. *Exp Clin Endocrinol Diabetes* 2012; **120**: 329-334 [PMID: 22231921 DOI: 10.1055/s-0031-1298016]
60 **Riaz S**, Skinner V, Srai SK. Effect of high dose thiamine on the levels of urinary protein biomarkers in diabetes mellitus type 2. *J Pharm Biomed Anal* 2011; **54**: 817-825 [PMID: 21130593 DOI: 10.1016/j.jpba.2010.11.008]
61 **Schupp N**, Dette EM, Schmid U, Bahner U, Winkler M, Heidland A, Stopper H. Benfotiamine reduces genomic damage in peripheral lymphocytes of hemodialysis patients. *Naunyn Schmiedebergs Arch Pharmacol* 2008; **378**: 283-291 [PMID: 18509620 DOI: 10.1007/s00210-008-0310-y]
62 **Stirban A**, Pop A, Fischer A, Heckermann S, Tschoepe D. Variability of skin autofluorescence measurement over 6 and 12 weeks and the influence of benfotiamine treatment. *Diabetes Technol Ther* 2013; **15**: 733-737 [PMID: 23964994 DOI: 10.1089/dia.2013.0103]
63 **Stirban A**, Pop A, Tschoepe D. A randomized, double-blind, crossover, placebo-controlled trial of 6 weeks benfotiamine treatment on postprandial vascular function and variables of autonomic nerve function in Type 2 diabetes. *Diabet Med* 2013; **30**: 1204-1208 [PMID: 23701274 DOI: 10.1111/dme.12240]

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REVIEW

Social determinants of type 2 diabetes and health in the United States

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Abstract

Diabetes is the sixth leading cause of death in the United States. To date, most research and resulting clinical strategies have focused on the individual with short-term health improvements that have not been maintained over time. Researchers more recently have recognized the need to consider the social determinants of diabetes and health along with individual factors. The purpose of this literature review is to examine current understanding of the social determinants affecting diabetes and health. A search of medical and nursing literature was conducted using PubMed, PsychInfo, CINAHL and MEDLINE databases, selecting articles published between 2000 and 2013. Search terms included: type 2 diabetes, social determinants, and health determinants. Inclusion criteria were: English language, human studies, social determinants of diabetes and health, and research in the United States. Additional search methods included reference chaining of the literature. Twenty research articles met the inclusion criteria for the review and analysis and included quantitative and qualitative methods. All studies selected for this review were descriptive in nature (n = 20). Fifteen studies were quantitative studies and five were qualitative studies. No intervention studies met inclusion criteria. Each study is summarized and critiqued. Study findings indicate that external or upstream factors consistently affect individuals diagnosed with diabetes, influencing self-management. Significant methodological limitations result directly from small sample sizes, convenience or nonprobability sampling, and low statistical power.

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Key words: Type 2 diabetes; Social determinants; Health determinants; Research; United States

Core tip: Social determinants of health and diabetes need to be considered when focusing on improving diabetes outcomes. Future research studies should focus on testing health outcomes of people with diabetes within the social determinants of health framework. Such research is particularly significant due to high rates of diabetes and subsequent disease sequelae.

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INTRODUCTION

Diabetes Mellitus affects approximately 25.6 million individuals or 11.3% of those over age 20. It is the sixth leading cause of death in the United States[1]. Diabetes places the individual at risk for serious long term complications including blindness, cardiovascular disease, end stage renal disease, hypertension, stroke, neuropathy, lower limb amputations, and premature death[1]. Estimated annual healthcare cost in 2012 for diabetes and its resulting complications was $245 billion[2]. Given the considerable differences internationally in methods of allocating health care resources, systems of funding and/or paying for care, and cultural attitudes to health and health care, the purpose of this review of the literature is to examine

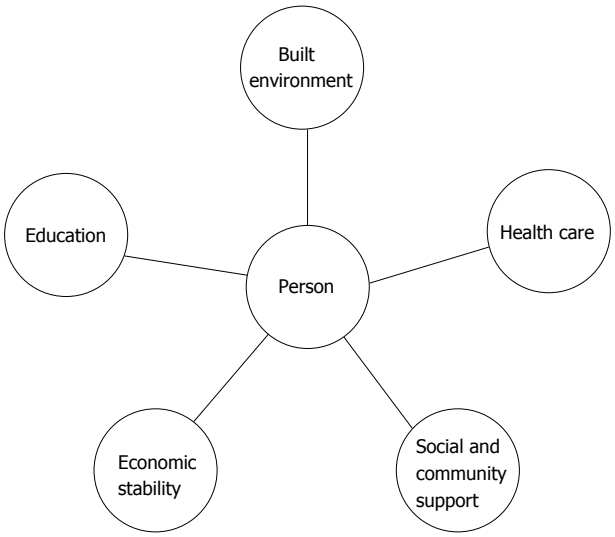

Figure 1 Social determinants influencing the individual's self-management of type 2 diabetes.

current understanding of the social determinants affecting diabetes and health in the United States, and to make recommendations for future research.

Historically, research and resulting clinical approaches focusing on the individual have led to improvement in self-management outcomes and reduction of cardiovascular risk factors; however, these short-term improvements have not been maintained over time. Researchers more recently have recognized the need to consider factors external to the individual, namely the social determinants of diabetes and health in order to achieve the goal of sustainable improvement in health outcomes[3,4]. For example, the United States government document Healthy People 2020 emphasizes the social and environmental factors that affect the individual and his/her health. A Healthy People 2020 goal for the diabetes health indicator is to "reduce the disease and economic burden of diabetes mellitus, and improve the quality of life for all persons who have, or at risk for diabetes"[5].

Social determinants of health are social-ecological factors affecting health[6]. The person, his/her social network, and cultural and environmental conditions form the overall framework. Constructs include external/environmental socio-ecological influences on the individual (Figure 1); for example, culture, environment, education, working conditions, access to medical care, and community infrastructure[5]. Therefore, external or upstream determinants such as social support and elements of the community affect the health of the individual. Specific socio-ecological factors identified from this literature review are examined below.

Built environment/community infrastructure

Components of the physical environment include factors such as transportation, neighborhood safety, and healthy food. When barriers to these factors are present to individuals with diabetes, inadequate access to resources among such disadvantaged populations means fewer resources are available to overcome barriers, thus effects are magnified[7-9]. For example, limited transportation in rural areas may require travel outside the local community to gain access to healthcare providers or access to healthy foods[6]. Urban residents may face transportation barriers such as lack of sidewalks[9], discouraging individuals from walking as a form of physical activity. Lack of public transportation in rural or urban areas can hinder travel for access to healthcare. Lack of neighborhood safety contributes to health disparities. An example of compounding factors is as follows: urban centers may have high crime rates with consequently fewer businesses and employment; reduced access to services including food and medical care; and diminished opportunity for outdoor activity including exercise[10]. Research has shown a relationship between improved health outcomes and access to healthy foods[11,12]. Emerging research in the area of nutraceuticals indicates that certain foods may provide health benefits to reduce disease process progression in diabetes and hyperlipidemia[12]. However, this relationship is a complicated one, as demonstrated by Jones-Smith *et al*[13] who found that, even with access to healthy food, socioeconomic status remains a strong predictor for obesity among African Americans diagnosed with diabetes.

Economic stability

Research has demonstrated a direct relationship between socio-economic status and health outcomes; however, other factors may explain a degree of variance in this relationship[14]. Zheng *et al*[14] found that education level, employment, and family income affect socioeconomic status and therefore health.

Education

Greater educational attainment has been linked with improved health outcomes[15] possibly because of a greater likelihood of socio-economic stability compared to those with lower levels of education. Other related factors may be the stability derived from marriage and/or a wider range of opportunities for better employment[15]. Moreover, research has shown that individuals with higher levels of education are more likely to participate in preventive healthcare including eating healthier (foods), being more physically active, and avoiding obesity[16].

Health care/access to medical care

Individuals may be subject to disparity in the availability of healthcare resources, including access to medical care, based on factors such as socioeconomic status, place of residence, race/ethnicity, and culture. Socioeconomic factors include educational level which in turn influences health insurance status[16]. Low income inner cities and remote rural regions often lack both primary and specialty healthcare providers, decreasing access to healthcare for inhabitants with chronic illnesses such as diabetes, hypertension, and cardiovascular disease. Absent or inadequate care may result in worsening or compounding of long-term effects of chronic diseases[17,18]. For example, recent research focusing on infants born preterm or with low

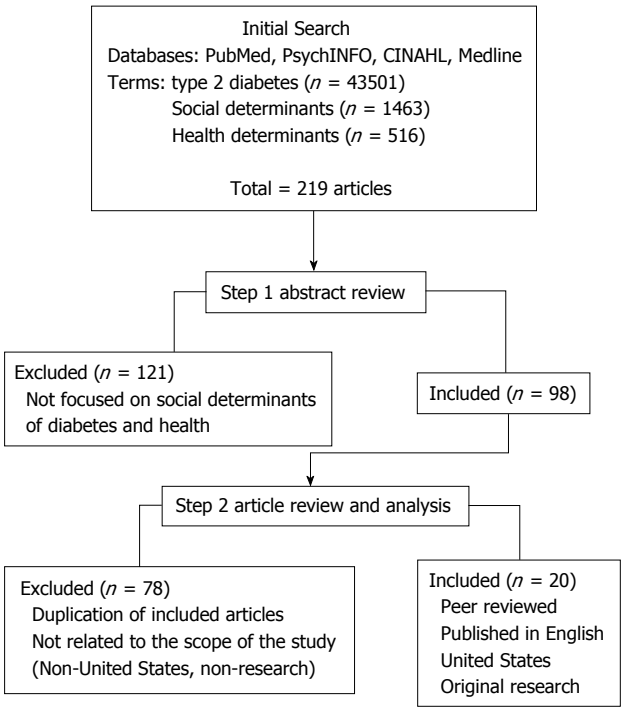

Figure 2 Manuscript selection for systematic review of the social determinants of diabetes and health.

birth weight demonstrates an increase in the development of insulin resistance and impaired glucose tolerance as adults[19,20]. Lower socioeconomic status may be associated with an individual's perception that lack of a collaborative patient-provider relationship is associated with improved diabetes outcomes[21].

Culture/social and community support

Social support includes individuals' "formal and informal relationships that give rise to a belief that one is cared for or supported emotionally in a defined situation such as working toward improving health outcomes"[22]. Degree of social support may vary between individuals and among ethnic groups; for example, research revealed that Hispanic individuals diagnosed with diabetes prefer group medical visits for self-management support whereas individuals from other ethnic groups have no preference[23]. Just as greater social support correlates with improved self-management outcomes, the perception of negative or low levels of social support has been shown to increase the risk of fewer self-management behaviors[24].

RESEARCH

A search of medical and nursing literature was conducted using PubMed, PsychInfo, CINAHL and MEDLINE databases. Additional search methods included reference chaining of the literature. Search terms included type 2 diabetes, social determinants and health determinants. Inclusion criteria were English language, human studies, social determinants of diabetes and health, and research in the United States. Exclusion criteria were type 1 diabetes, reviews, and studies not focusing primarily on social determinants of diabetes and health; for example, biomarkers. The initial search of the literature retrieved 59036 articles on type 2 diabetes; 12871 articles on social determinants; 14866 articles on health determinants. Sixty one duplicate articles, one book review brief, one editorial commentary, and two conference proceeding abstracts were also excluded (Figure 2). Twenty articles met criteria for the review (Table 1).

Twenty articles met the inclusion criteria for the review and analysis. All studies selected for this review were descriptive in nature (n = 20). Fifteen studies were quantitative studies and five were qualitative studies[25-29,32-44]. Although sample size ranged from 15 to 81917 participants, many samples were fewer than one hundred subjects. All studies focused on individuals diagnosed with diabetes. There were no interventional or randomized control trial studies. The majority were cross-sectional, collecting data only once. For quantitative studies, two were mixed methods, including a survey and interview; five were secondary data analysis, and eight were surveys. Qualitative studies used either focus groups or individual semi-structured interviews (n = 5). Fourteen studies focused on social determinants from the patient or client perspective; three studies focused both on staff/healthcare provider and patient/client, while three studies viewed social determinants of health from the perspective of the healthcare provider alone. All studies focused on one or more of the constructs of social determinants of health: built environment, economic stability, health care, or culture/social support.

Built environment/community infrastructure

Authors of four articles discussed the built environment and community infrastructure. Research studies used purposive sampling, limiting the generalizability of findings to other populations. Three studies focused on populations known to have a disproportionate burden of type 2 diabetes, including African Americans and Hispanic/Latino. The built environment was a stronger predictor of health outcomes than race. Three studies[25-27] reported on upstream social determinants and the influence on food environments for at risk immigrant Hispanic population. Findings included high rates of poverty with 60% of living below United States definition of poverty and 40% living at 170% below federal poverty level. Educational attainment was less than the United States average with 80% of individuals not entering college. Thirty-three percent had not completed elementary school. In comparison the national United States rate of high school completion is 89.9% in 2010[25]. One study focused on Asian Americans. No studies included American Indians or Pacific Islanders. Two studies were community-based, focusing on food environment and access to healthy food. Transportation was discussed in three articles as a barrier to access both healthcare and healthy food. Research participants reported lack of access to quality, quantity, and

Table 1 Summary of reviewed studies

Ref.	Purpose	Design/method	Sample	Findings	Strengths/limitations/implications
Chaufan *et al*[25] (2011)	To examine "upstream" social determinants of health in a Latino immigrant population focusing on T2DM risk and food environment	Mixed methods focus groups, Food store survey instrument California	Staff members (n = 6); clients (n = 15)	Poverty prevented Latino immigrants from access to adequate housing, high quality education, and culturally appropriate food choices	Community-based interviews elicited lived experience of Latino immigrant population Limitations: small sample size, nonrandomized, descriptive study Implications: Policy change to enable access to affordable culturally acceptable healthy foods
Carbone *et al*[26] (2007)	To describe factors influencing Latino (Puerto Rican) diabetes self-management	Qualitative-focus groups Health center in Massachusetts, United States	Healthcare providers (n = 15) Patients (n = 37)	Cultural influence of family through traditional gender roles; financial restraints Social network (family and friends) source of strength; spirituality and faith important; discrepancy between healthcare provider and patient focus in self-management	Community-based limitations: Small sample size, qualitative study, not generalizable to all Latino populations Implications: account for inclusion of family and social networks in diabetes self-management education, need for increased cultural awareness by Healthcare provider of influencing factors on patient self-management
Chaufan *et al*[27] (2011)	To gain understanding how food environments influence a low income immigrant population	Qualitative-focus groups	Staff (n = 6) Clients (n = 15)	Barriers to food access: transportation, language barriers, poverty, employment	Strengths: community based Limitations: small sample size, not generalizable Implications: Need for policy to increase food access for immigrant populations in lower socio-economic levels
Tjia *et al*[28] (2008)	To gain understanding of barriers to medication adherence among older adults with T2DM	Qualitative-semi structured interviews	Adults over age 65 (n = 22) Female n = 16	Older adults with T2DM had concerns about medication cost or medication burden but did not discuss with physician	Strengths: perspective of older adults Limitations: small sample size, not generalizable Implications: need for increased patient-physician communication; policy level for messages to encourage open patient-physician dialogue for medical treatment
Denham *et al*[29] (2010)	To explore perceived patient barriers to diabetes education among healthcare providers	Cross-sectional survey	Healthcare providers from three practice settings: federally qualified healthcare centers; health departments; clinics (n = 182)	Perceived barriers: transportation; fewer diabetes educators and physicians; lack of insurance; education materials not screened for literacy levels nor cultural appropriateness	Strengths: look at perceived barriers to diabetes education among healthcare providers Limitations: small sample size, multiple sites, sample bias Implications: policy need to increase diabetes education reimbursement to providers for all individuals nationally; need to screen materials for literacy levels
Heuer *et al*[30] (2006)	To describe Hispanic migrant farmworkers perceptions of diabetes	Qualitative phenomenological study	Migrant farmworkers (n = 12) Female n = 6	Cultural/folk beliefs that diabetes was caused by stress or emotions	Strengths: focused on hispanic explanatory model of diabetes Limitations: small sample size, not generalizable Implications: need to tailor diabetes education to address culture and health beliefs
Shigaki *et al*[31] (2010)	To examine how patients diagnosed with type 2 diabetes view role of nurses in disease management	Qualitative Semi-structured individual interviews	n = 13 Female n = 7 White n = 9 African American n = 3 Other n = 1	Nurse viewed as a positive partner in disease management, patients prefer team-based medical care, open communication between healthcare providers and patient	Strengths: Patient perspective on nurse as partner in healthcare Limitation: small sample size; sample bias Implications: Nurse partner may provide link between patient and improving health outcomes in individuals with multiple co-morbidities including diabetes
Fitzgerald *et al*[32] (2008)	To determine diabetes care perceptions in patient and provider	Quantitative cross sectional survey	Providers n = 71 Patients: n = 273 female 61% White 63% African American 33%	Patient and provider differences included patients with more positive attitudes family support, paying for diabetes care	Strengths: quantitative study using reliable and valid instrument Limitations: may not be representative for patients with no co-morbidities Implications: Importance of communication between patient and provider and not making assumptions about patients

Zulkowski *et al*[33] (2005)	To examine differences between patient self-report and provider documentation in rural health center	Quantitative cross sectional survey; medical record review	*n* = 149 Female *n* = 86	Statistical difference between patient and provider on medical management or diabetes knowledge; most patients had not been seen by diabetes educator or dietician	Strengths: good response rate (61%); limitations: race not documented Implications: Need for increased access to diabetes education in rural areas; need for open communication between patient and provider
Ford *et al*[34] (2002)	To determine relationship between socio-economic status and race on diabetes management	Quantitative-random sampling by socio-economic status and race	*n* = 50 African American *n* = 21 Caucasian *n* = 29 Female *n* = 22	Differences in perception of sense of loss by race but not socio-economic status	Strengths: Limitations: small sample size: not generalizable; underpowered ($\alpha = 0.34$) Implications: Need for education to stress management to prevent disease related complications
Mani *et al*[35] (2011)	To determine the influence of social networks on diabetes self-management	Cross-sectional survey	*n* = 154 Female *n* = 88 White *n* = 78 African American *n* = 61	Diabetes concern increased when larger social network diagnosed with diabetes	Strengths: patient perspective of importance of social networks Limitations: not generalizable-sample from two inner city clinics, only English speaking, data skewed Implications: Important to consider the influence of social networks on diabetes self-management
Song *et al*[36] (2012)	To examine social networks among Korean Americans diagnosed with type 2 diabetes	Cross-sectional survey from a larger study	*n* = 83 Female *n* = 35 Married *n* = 73	Gender differences in source of support, men sought spouse, women had higher unmet needs for social support than men, self-efficacy negatively associated with unmet social support, education level strong predictor of self-care activities, unmet needs for social support negatively associated with diabetes self-care	Strengths: patient perception of social support Limitations: small homogenous sample; not generalizable Implications: need to recognize the role of gender in determining social support among Korean Americans
Kollannoor-Samuel *et al*[37] (2011)	To identify influence of social determinants of health on FPG and HbA1c among low income Latinos	Cross-sectional	*n* = 211 Female *n* = 155 Puerto Rican *n* = 171 Unemployed *n* = 178 Spanish speaking only *n* = 138	Lower socio-economic status had higher FPG and HbA1c levels; better long term glycemic control when insured; increased physical activity associated with lower FPG and HbA1c levels	Strengths: sample size; part of larger RCT Limitations: large number of female participants-not generalizable or transferable to other Latino populations; unknown length of time since diagnosis Implications: Importance of physical activity for improved glycemic control; need for health insurance, increase in education to improve glycemic outcomes over the long term
Chiu *et al*[38] (2011)	To determine how gender influences functional limitations with type 2 diabetes	Secondary data analysis	*n* = 1619 Female *n* = 861	Psychosocial factors mediator between biological and exercise factors	Strengths: consistent with previous research Limitations: participant self report; no causal pathway Implications: Need to incorporate gender and psychosocial factors in diabetes treatment plan
Iida *et al*[39] (2010)	Examine role of spousal support for individual diagnosed with type 2 diabetes	Mixed methods-longitudinal Computer diary survey; individual interviews	*n* = 129 married couples Caucasian 75%; African American 23.6%	Physical symptoms increased spousal support; women gave higher level of support when spouse worried about diabetes; less spousal support when negative emotional effect from previous day	Strengths: sample size Longitudinal study, diary Limitations: unable to determine causal effect Implications: Need to measure and include spousal support in diabetes self-management plan

Mayberry *et al*[40] (2005)	To assess determinants of glycemic monitoring by primary care provider among medicaid beneficiaries	Retrospective cohort study	*n* = 3321 African American (2025) 61%; White (1296) 39%; Female 74.6%; Urban 49.6%; rural 50.4%	African Americans diagnosed at younger age; frequency of physician visits and medication prescription strong predictor of meeting ADA recommendation guideline for HbA1c monitoring; low monitoring of HbA1c and FPG among medicaid beneficiaries with type 2 diabetes, no significance between Black and White Medicaid beneficiaries for diabetes monitoring	Strengths: provides insight into determinants of glucose monitoring in Medicaid beneficiaries Limitations: Secondary data analysis; inability to determine patient-physician relationship Implications: Need to establish ADA evidence guidance protocol in office practice settings
Ford *et al*[41] (2005)	To determine estimates of obesity and diabetes weight information from healthcare providers in 100 United States metropolitan service areas	Cross sectional survey: 2000 Behavioral Risk Factor Surveillance System	*n* =81917 individuals with BMI data Sample size varied due to missing data	Highest prevalence of obesity Appalachian region; OR of being diagnosed with diabetes 3.5 higher in Charleston WV than Santa Fe NM; weight loss or maintenance discussed with physician 11.7%-34.6%	Strength: study at community level Limitation: secondary data analysis, missing data; self report Implications: Policy level- need to examine determinants of obesity and diabetes at local metropolitan service areas. Healthcare providers need to include discussion of weight management for individuals diagnosed with obesity or diabetes
Adams *et al*[42] (2008)	To examine medication adherence as a factor of glycemic control based on race	Secondary data analysis newly diagnosed patients prescribed oral medications	*n* = 1806 Black *n* = 467 White *n* = 1339	Black patients higher A1c at diagnosis; insufficient evidence to determine medication adherence by race	Strengths: limitations: unable to determine causal effect; potential overestimation of medication adherence based on data; implications: Need at policy level to provide screening for earlier diagnosis of Black and female patients
Aikens *et al*[43] (2005)	To examine the relationship of patient-provider communication on diabetes self-management and outcomes	Cross-sectional Telephone survey	*n* = 736 White 51% Black 20% Hispanic 11.9% Female 31.6%	Primary care provider main manager of diabetes (70.9%) General patient-provider communication related to improved quality of life; diabetes specific patient-provider communication related to glycemic control	Strengths: sample across three health systems; limitations: cross-sectional study; self-report; implications: need for open patient-provider communication to enhance problem solving, need to tailor management plan to individual patient
Paris *et al*[44] (2001)	To determine if determinants of type 2 diabetes were present in personnel entering the military	Cross-sectional secondary data analysis	Diagnosed with diabetes *n* = 419 Not diagnosed with diabetes *n* = 627	Enlisted military rank as socioeconomic measure for diabetes diagnosis higher in lower rank, minorities higher level of diabetes; educational not significant variable	Strengths: adequate power Limitations: no causal effect; potential for misclassification of diabetes diagnosis in database; implications: need for focus on policy level for increased physical activity and body mass index monitoring

FPG: Fasting plasma glucose; HbA1c: Glycosylated hemoglobin A1c; BMI: Body mass index; T2DM: Type 2 diabetes mellitus; RCT: Randomized control trial; ADA: American diabetes Association.

culturally-acceptable food choices[25-27]. Study limitations include small sample size and descriptive statistics.

Economic stability

Five articles focused on economic stability. Three studies were cross-sectional survey, one involved focus group interviews, and one was a secondary analysis. Sample size in these studies ranged from 50 to 419. Discussion focused on health insurance, financial barriers, poverty, and affordability of medication from the patient or client perspective. One study focused on indirect economic factors, such as military rank, as a predictor of diabetes diagnosis[44]. Two studies compared patient/client perspectives to healthcare provider perceptions of economic barriers to diabetes self-management[25,32]. Three of the five studies compared race as a factor in economic stability within Latino and African

American populations[26,34,37]. Findings included individual acknowledgement that economic distress in diabetes self-management was important however, factors were also identified as sources of additional strength for individuals diagnosed with diabetes. Sources of support included culture and/or social support[26,34]. The influences of economic factors by race/ethnicity on diabetes outcomes were non-conclusive. Therefore, economic stability may be a strong determinant of diabetes and health regardless of race/ethnicity. Studies focused on target populations, limiting to selected urban regions for study.

Health care/access to medical care

There were nine studies found in which researchers examined the role of health care and/or access to medical care within the social determinants of diabetes and health framework. One study compared patients' and healthcare providers' perspectives on diabetes management[32]. Another research report examined healthcare providers' perception of patient barriers to diabetes management[29]. The remaining seven articles focused on the patient's perceptions of healthcare related to diabetes management and barriers to care. Sample size for the patient-only studies ranged from 13 to 81917. Eight studies were cross-sectional descriptive in design, and one was a secondary data analysis from the Behavioral Risk Factor Surveillance System. Most researchers reported that patients viewed their health in a more positive light than did providers based on medical record reviews. The concerns of healthcare provider included the costs associated with diabetes management[29,32]. Patient-provider communication varied among patients. Three articles focused on positive health outcomes with open patient-provider communication[31,40,43]. One article described physicians as often initiating communication about medication adherence, whereas patients were hesitant to initiate communication with physicians relating to medication burden and costs[28]. This may, in part, explain perceived lack of patient medication adherence which increases the potential for poorer health outcomes. One qualitative study described patients' preference for diabetes care teams in which the team's link between patient and physician was a nurse[31]. Two studies demonstrated increased quality of life and better glycemic control with positive patient-provider communication[40,43]. However, when looking at diabetes prevention and knowledge, two studies reported the need for provision of diabetes education focusing on basic management and the need for discussion of weight management or weight loss for diabetes prevention[33,41].

Culture/social and community support

Seven articles met the inclusion criteria focusing on the constructs of culture and community support. Four of the seven researchers reported on cross-sectional surveys, one study involved focus groups in a community setting, one study used a phenomenological method of analysis, and one used mixed methods incorporating a computer diary and individual interviews. Two of the seven articles included healthcare provider perceptions. Of these two articles, one had a sample of both patient and healthcare provider. Sample size for the seven articles ranged from 12 to 273. Two articles focused on cultural determinants of diabetes and health in Latino/Hispanic populations. Cultural beliefs in Hispanic populations included the belief that diabetes was caused by increased stress[30]. The authors noted that the discovery of this belief provides an opportunity for healthcare providers or trusted community sources to provide education to increase diabetes knowledge. Three articles focused on the traditional roles of gender and culture, whereby married women provided increased support to their spouse when he voiced concerns about diabetes and health[26,36,39]. One article focusing on Korean Americans found that women had an increase in unmet needs when providing support for their spouses, which negatively affected their diabetes self-care[36]. Two articles discussed social support or social networks as positive influences for diabetes self-management and health[26,32]. However, one article described African American patients' concern about their diabetes management and health when multiple members of their social network were diagnosed with diabetes or experienced complications of diabetes[35]. One article discussed healthcare providers' perceived barriers in rural healthcare settings[29], pointing out an apparent lack of culturally appropriate educational materials within healthcare clinic settings.

CONCLUSION

This critique of the literature about social determinants of diabetes and health focused on research of United States populations published between 2000 and 2013. A total of 20 research studies met established criteria. All 20 studies identified for this review were descriptive. The majority of studies were published in journals with a focus on public health or nursing. Results of this review are useful for health professionals who develop programs and/or interventions for people diagnosed with diabetes because evidence indicates that social determinants affect patient adherence, effectiveness of treatments, and overall health outcomes.

Study findings indicate that external or upstream factors prominently affect individuals diagnosed with diabetes, in part by influencing self-management and in turn exerting lasting effects on long-term diabetes and health outcomes. The most significant methodological limitations of the studies examined result directly from small sample size, convenience or nonprobability sampling, and low statistical power. Methodological limitations of studies included in this review also include a lack of intervention studies. Future research needs to include community-based intervention studies focusing on the reduction of diabetes disparities and improvement of health outcomes within the social determinants of health framework. Such research is particularly needed given the high rates of diabetes and subsequent disease sequelae. Cultural tailoring

of diabetes prevention educational materials and cultural tailoring of education in group settings may afford the means to increase patients' knowledge of the disease for earlier diagnosis and earlier intervention to prevent diabetes complications. Encouragement of spousal support within the construct of acknowledging cultural norms may provide a means for improving diabetes outcomes and health. The influence of social determinants of health on diabetes outcomes needs to be tested in intervention studies to provide a foundation for effective interventions to impact the current epidemic of diabetes in the United States and around the globe. Prospective interventional studies evaluating the influence of social determinants will be key to lay a foundation for effective interventions and improvement of diabetes and health outcomes.

REFERENCES

1 **Centers for Disease Control and Prevention.** Diabetes Report Card 2012. Atlanta, GA: US Department of Health and Human Services, 2012
2 **Dall TM**, Zhang Y, Chen YJ, Quick WW, Yang WG, Fogli J. The economic burden of diabetes. *Health Aff* (Millwood) 2010; **29**: 297-303 [PMID: 20075080 DOI: 10.1377/hlthaff.2009.0155]
3 **Hill J**, Nielsen M, Fox MH. Understanding the social factors that contribute to diabetes: a means to informing health care and social policies for the chronically ill. *Perm J* 2013; **17**: 67-72 [PMID: 23704847]
4 **Liburd LC**, Jack L, Williams S, Tucker P. Intervening on the social determinants of cardiovascular disease and diabetes. *Am J Prev Med* 2005; **29**: 18-24 [PMID: 16389121 DOI: 10.1016/j.amepre.2005.07.013]
5 Healthy People 2020. US Department of Health and Human Services. Available from: URL: http: //www.healthypeople.gov/2020/topicsobjectives2020/objectiveslist.aspx?topicId=8
6 **Braveman P**, Egerter S, Williams DR. The social determinants of health: coming of age. *Annu Rev Public Health* 2011; **32**: 381-398 [PMID: 21091195 DOI: 10.1146/annurev-publhealth-031210-101218]
7 **Hutch DJ**, Bouye KE, Skillen E, Lee C, Whitehead L, Rashid JR. Potential strategies to eliminate built environment disparities for disadvantaged and vulnerable communities. *Am J Public Health* 2011; **101**: 587-595 [PMID: 21389288 DOI: 10.2105/AJPH.2009.173872]
8 **DeGuzman PB**, Kulbok PA. Changing health outcomes of vulnerable populations through nursing's influence on neighborhood built environment: a framework for nursing research. *J Nurs Scholarsh* 2012; **44**: 341-348 [PMID: 23062005 DOI: 10.1111/j.1547-5069.2012.01470.x]
9 **Hood E**. Dwelling disparities: how poor housing leads to poor health. *Environ Health Perspect* 2005; **113**: A310-A317 [PMID: 15866753 DOI: 10.1289/ehp.113-a310]
10 **Rashid JR**, Spengler RF, Wagner RM, Melanson C, Skillen EL, Mays RA, Heurtin-Roberts S, Long JA. Eliminating health disparities through transdisciplinary research, cross-agency collaboration, and public participation. *Am J Public Health* 2009; **99**: 1955-1961 [PMID: 19762652 DOI: 10.2105/AJPH.2009.167932]
11 **Matson-Koffman DM**, Brownstein JN, Greany ML. A site-specific literature review of policy and environmental interventions that promote physical activity and nutrition for cardiovascular health: what works? *Am J Health Promot* 2005; **19**: 167-193 [PMID: 15693346 DOI: 10.4278/0890-1171-19.3.167]
12 **Scicchitano P**, Cameli M, Maiello M, Modesti PA, Muiesan NL, Novo S. Nutraceuticals and dyslipidaemia: Beyond the common therapeutics. *J Funct Foods* 2014; **6**: 11-32 [DOI: 10.1016/j.jff.2013.12.006]
13 **Jones-Smith JC**, Karter AJ, Warton EM, Kelly M, Kersten E, Moffet HH, Adler N, Schillinger D, Laraia BA. Obesity and the food environment: income and ethnicity differences among people with diabetes: the Diabetes Study of Northern California (DISTANCE). *Diabetes Care* 2013; **36**: 2697-2705 [PMID: 23637355 DOI: 10.2337/dc12-2190]
14 **Zheng H**, George LK. Rising U.S. income inequality and the changing gradient of socioeconomic status on physical functioning and activity limitations, 1984-2007. *Soc Sci Med* 2012; **75**: 2170-2182 [PMID: 22959768 DOI: 10.1016/j.socscimed.2012.08.014]
15 **Montez JK**, Zajacova A. Explaining the widening education gap in mortality among U.S. white women. *J Health Soc Behav* 2013; **54**: 166-182 [PMID: 23723344 DOI: 10.1177/0022146513481230]
16 **Pampel FC**, Krueger PM, Denney JT. Socioeconomic Disparities in Health Behaviors. *Annu Rev Sociol* 2010; **36**: 349-370 [PMID: 21909182 DOI: 10.1146/annurev.soc.012809.102529]
17 **Behringer B**, Friedell GH. Appalachia: where place matters in health. *Prev Chronic Dis* 2006; **3**: A113 [PMID: 16978488]
18 **Murray CJ**, Kulkarni S, Ezzati M. Eight Americas: new perspectives on U.S. health disparities. *Am J Prev Med* 2005; **29**: 4-10 [PMID: 16389119]
19 **Ciccone MM**, Scicchitano P, Salerno C, Gesualdo M, Fornarelli F, Zito A, Filippucci L, Riccardi R, Cortese F, Pini F, Angrisani L, Di Mauro A, Schettini F, Laforgia N. Aorta structural alterations in term neonates: the role of birth and maternal characteristics. *Biomed Res Int* 2013; **2013**: 459168 [PMID: 23984364 DOI: 10.1155.2013/459168]
20 **Ingelfinger JR**, Nuyt AM. Impact of fetal programming, birth weight, and infant feeding on later hypertension. *J Clin Hypertens (Greenwich)* 2012; **14**: 365-371 [PMID: 22672090 DOI: 10.1111/j.1751-7176.2012.00660.x]
21 **Nam S**, Chesla C, Stotts NA, Kroon L, Janson SL. Barriers to diabetes management: patient and provider factors. *Diabetes Res Clin Pract* 2011; **93**: 1-9 [PMID: 21382643 DOI: 10.1016/j.diabres.2011.02.002]
22 **Strom JL**, Egede LE. The impact of social support on outcomes in adult patients with type 2 diabetes: a systematic review. *Curr Diab Rep* 2012; **12**: 769-781 [PMID: 22949135 DOI: 10.1007/s11892-012-0317-0]
23 **Sarkar U**, Piette JD, Gonzales R, Lessler D, Chew LD, Reilly B, Johnson J. Preferences for self-management support: findings froma survey of diabetes patients in safety-net health systems. *Patient Educ Couns* 2008; **34**: 1037-1044
24 **Tang TS**, Brown MB, Funnell MM, Anderson RM. Social support, quality of life, and self-care behaviors amongAfrican Americans with type 2 diabetes. *Diabetes Educ* 2008; **34**: 266-276 [PMID: 18375776 DOI: 10.1177/0145721708315680]
25 **Chaufan C**, Davis M, Constantino S. The twin epidemics of poverty and diabetes: understanding diabetes disparities in a low-income Latino and immigrant neighborhood. *J Community Health* 2011; **36**: 1032-1043 [PMID: 21533887 DOI: 10.1007/s10900-011-9406-2]
26 **Carbone ET**, Rosal MC, Torres MI, Goins KV, Bermudez OI. Diabetes self-management: perspectives of Latino patients and their health care providers. *Patient Educ Couns* 2007; **66**: 202-210 [PMID: 17329060 DOI: 10.1016/j.pec.2006.12.003]
27 **Chaufan C**, Constantino S, Davis M. 'It's a full time job being poor': understanding barriers to diabetes prevention in immigrant communities in the USA. *Pub Health* 2012; **22**: 147-158 [DOI: 10.1080/09581596.2011.630383]
28 **Tjia J**, Givens JL, Karlawish JH, Okoli-Umeweni A, Barg FK. Beneath the surface: discovering the unvoiced concerns of older adults with type 2 diabetes mellitus. *Health Educ Res* 2008; **23**: 40-52 [PMID: 17272292 DOI: 10.1093/her/cyl161]
29 **Denham SA**, Remsburg K, Wood L. Diabetes education in the Appalachian region: providers' views. *Rural Remote Health* 2010; **10**: 1321 [PMID: 20560683]

30 **Heuer L**, Lausch C. Living with diabetes: perceptions of Hispanic migrant farmworkers. *J Community Health Nurs* 2006; **23**: 49-64 [PMID: 16445364 DOI: 10.1207/s15327655jchn2301_5]

31 **Shigaki CL**, Moore C, Wakefield B, Campbell J, LeMaster J. Nurse partners in chronic illness care: patients' perceptions and their implications for nursing leadership. *Nurs Adm Q* 2010; **34**: 130-140 [PMID: 20234247 DOI: 10.1097/NAQ.0b013e3181d917bd]

32 **Fitzgerald JT**, Stansfield RB, Tang T, Oh M, Frohna A, Armbruster B, Gruppen L, Anderson R. Patient and provider perceptions of diabetes: measuring and evaluating differences. *Patient Educ Couns* 2008; **70**: 118-125 [PMID: 17997265 DOI: 10.1016/j.pec.2007.09.005]

33 **Zulkowski K**, Coon P. Patient perceptions and provider documentation of diabetes care in rural areas. *Ostomy Wound Manage* 2005; **51**: 50-58 [PMID: 15984399]

34 **Ford ME**, Havstad SL, Brooks BL, Tilley BC. Perceptions of diabetes among patients in an urban health care system. *Ethn Health* 2002; **7**: 243-254 [PMID: 12772544]

35 **Mani N**, Caiola E, Fortuna RJ. The influence of social networks on patients' attitudes toward type II diabetes. *J Community Health* 2011; **36**: 728-732 [PMID: 21298473 DOI: 10.1007/s10900-011-9366-6]

36 **Song Y**, Song HJ, Han HR, Park SY, Nam S, Kim MT. Unmet needs for social support and effects on diabetes self-care activities in Korean Americans with type 2 diabetes. *Diabetes Educ* 2016; **38**: 77-85 [PMID: 22222514 DOI: 10.1177/0145721711432456]

37 **Kollannoor-Samuel G**, Chhabra J, Fernandez ML, Vega-López S, Pérez SS, Damio G, Calle MC, D'Agostino D, Pérez-Escamilla R. Determinants of fasting plasma glucose and glycosylated hemoglobin among low income Latinos with poorly controlled type 2 diabetes. *J Immigr Minor Health* 2011; **13**: 809-817 [PMID: 21181446 DOI: 10.1007/s10903-010-9428-3]

38 **Chiu CJ**, Wray LA. Gender differences in functional limitations in adults living with type 2 diabetes: biobehavioral and psychosocial mediators. *Ann Behav Med* 2011; **41**: 71-82 [PMID: 20827519 DOI: 10.1007/s12160-010-9226-0]

39 **Iida M**, Parris Stephens MA, Rook KS, Franks MM, Salem JK. When the going gets tough, does support get going? Determinants of spousal support provision to type 2 diabetic patients. *Pers Soc Psychol Bull* 2010; **36**: 780-791 [PMID: 20445023 DOI: 10.1177/0146167210369897]

40 **Mayberry RM**, Davis T, Alema-Mensah E, Samadi AR, Finley R, Jones A. Determinants of glycemic status monitoring in Black and White Medicaid beneficiaries with diabetes mellitus. *J Health Care Poor Underserved* 2005; **16**: 31-49 [PMID: 16327095 DOI: 10.1353/hpu.2005.0128.]

41 **Ford ES**, Mokdad AH, Giles WH, Galuska DA, Serdula MK. Geographic variation in the prevalence of obesity, diabetes, and obesity-related behaviors. *Obes Res* 2005; **13**: 118-122 [PMID: 15761170 DOI: 10.1038/oby.2005.15]

42 **Adams AS**, Trinacty CM, Zhang F, Kleinman K, Grant RW, Meigs JB, Soumerai SB, Ross-Degnan D. Medication adherence and racial differences in A1C control. *Diabetes Care* 2008; **31**: 916-921 [PMID: 18235050 DOI: 10.2337/dc07-1924.]

43 **Aikens JE**, Bingham R, Piette JD. Patient-provider communication and self-care behavior among type 2 diabetes patients. *Diabetes Educ* 2005; **31**: 681-690 [PMID: 16203852 DOI: 10.1177/0145721705280829]

44 **Paris RM**, Bedno SA, Krauss MR, Keep LW, Rubertone MV. Weighing in on type 2 diabetes in the military: characteristics of U.S. military personnel at entry who develop type 2 diabetes. *Diabetes Care* 2001; **24**: 1894-1898 [PMID: 11679453]

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REVIEW

Novel and emerging diabetes mellitus drug therapies for the type 2 diabetes patient

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Abstract

Type 2 diabetes mellitus is a metabolic disorder of deranged fat, protein and carbohydrate metabolism resulting in hyperglycemia as a result of insulin resistance and inadequate insulin secretion. Although a wide variety of diabetes therapies is available, yet limited efficacy, adverse effects, cost, contraindications, renal dosage adjustments, inflexible dosing schedules and weight gain significantly limit their use. In addition, many patients in the United States fail to meet the therapeutic HbA1c goal of < 7% set by the American Diabetes Association. As such new and emerging diabetes therapies with different mechanisms of action hope to address some of these drawbacks to improve the patient with type 2 diabetes. This article reviews new and emerging classes, including the sodium-glucose cotransporter-2 inhibitors, 11β-Hydroxysteroid dehydrogenase type 1 inhibitors, glycogen phosphorylase inhibitors; protein tyrosine phosphatase 1B inhibitors, G Protein-Coupled receptor agonists and glucokinase activators. These emerging diabetes agents hold the promise of providing benefit of glucose lowering, weight reduction, low hypoglycemia risk, improve insulin sensitivity, pancreatic β cell preservation, and oral formulation availability. However, further studies are needed to evaluate their safety profile, cardiovascular effects, and efficacy durability in order to determine their role in type 2 diabetes management.

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Key words: Type 2 diabetes mellitus; Sodium dependent glucose co-transporter 2 inhibitors; 11β-Hydroxysteroid dehydrogenase type 1 inhibitors; Glycogen phosphorylase inhibitors; Protein tyrosine phosphatase 1B inhibitors; G protein-coupled receptor agonists; Glucokinase activators

Core tip: Type 2 diabetes mellitus is a metabolic disorder of deranged fat, protein and carbohydrate metabolism resulting in hyperglycemia. Limited efficacy, adverse effects, cost, contraindications, renal dosage adjustments, inflexible dosing schedules and weight gain significantly limit the use of currently available anti-hyperglycemic agents. In the past, drug researchers targeted defects of pancreatic β-cell failure and insulin resistance, but more recent attention has shifted to other contributing factors. This article reviews new and emerging diabetes classes, including the sodium-glucose cotransporter-2 inhibitors, 11β-Hydroxysteroid dehydrogenase type 1 inhibitors, glycogen phosphorylase inhibitors, protein tyrosine phosphatase 1B inhibitors, G protein-coupled receptor agonists, and glucokinase activators.

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litus drug therapies for the type 2 diabetes patient. *World J Diabetes* 2014; 5(3): 305-315 Available from: URL: http://www.wjgnet.com/1948-9358/full/v5/i3/305.htm DOI: http://dx.doi.org/10.4239/wjd.v5.i3.305

INTRODUCTION

Type 2 diabetes mellitus is a metabolic disorder of deranged fat, protein and carbohydrate metabolism resulting in hyperglycemia from insulin resistance and inadequate insulin secretion, which can cause complications of nephropathy, retinopathy, neuropathy, and cardiovascular disorders[1,2].

Diabetes mellitus is an epidemic in the United States and the world. According to the International Diabetes Federation's 2013 statistics, 382 million people worldwide have diabetes, which is estimated to increase to 592 million by 2035[3]. The Centers for Disease Control and Prevention estimates 79 million Americans have pre-diabetes and approximately 26 million have diabetes mellitus of which seven million of these are still undiagnosed[4].

Despite a wide variety of available food and drug association (FDA) approved oral and injectable diabetes therapies, limited efficacy, adverse effects, cost, contraindications, renal dosage adjustments, inflexible dosing schedules and weight gain significantly limit their use[5,6].

In addition, less than 50% of patients with type 2 diabetes in the United States achieve the HbA1c goal of < 7% set by the American Diabetes Association[7].

Currently available oral agent classes include sulfonylureas, meglitinides, biguanide, α-glucosidase inhibitors, dipeptidyl peptidase-4 (DPP-4) inhibitors, dopamine agonist, bile acid sequestrant, thiazolidinediones and their combinations. Injectable agents include insulin, amylin analogue and incretin mimetics.

In the past, drug researchers and manufacturers targeted the primary pathophysiologic defects in type 2 diabetes of pancreatic β-cell failure and insulin resistance, but more recent attention has shifted to other contributing factors including increased glucose reabsorption by the kidneys, and the contributing effects to hyperglycemia by glucagon, glucocorticoid, glycogen, 11β-Hydroxysteroid dehydrogenase-2 and others. As such new and emerging diabetes therapies with new mechanisms of action hope to address these contributing pathophysiologic defects and offer new approaches in order for the patient to achieve therapeutic goals[1,6]. Table 1 lists the new and emerging drug therapy and approaches[8].

An ideal antihyperglycemic agent will be a safe, tolerable, efficacious, cost effective oral agent with a flexible dosage schedule providing clinically significant weight loss with cardiovascular and mortality benefits. This article reviews several new classes of antihyperglycemic agents, including the sodium-glucose cotransporter-2 inhibitors (which are furthest along in development); 11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD-1) inhibitors, glycogen phosphorylase inhibitors, protein tyrosine phosphatase 1B inhibitors, G Protein-Coupled receptor agonists and glucokinase (GK) activators.

SODIUM DEPENDENT GLUCOSE CO-TRANSPORTER 2 INHIBITORS

Kidney and sodium dependent glucose co-transporter 2 transporters

Glucose homeostasis involves the liver, pancreas and the kidney[9]. Glucose transporter proteins (GLUT) and sodium-dependent glucose co-transporters (SGLT) are responsible for glucose transportation across the plasma membrane into cells[10].

Over the course of 24 h, the kidney filters 180 g of glucose while only 500 mg is excreted in the urine, and the rest is reabsorbed as it flows from the glomerulus to the proximal convoluted tubules then to the bloodstream[10]. GLUTs and SGLTs are involved in this glucose reabsorption and active transportation of glucose across cell membranes against concentration gradients[10,11].

SGLT-1 is responsible for 10% of glucose uptake and is expressed in the heart, skeletal muscle, gastrointestinal tract, liver, lung and the S3 segment of the proximal tubule of the kidney, while SGLT-2 is responsible for 90% of glucose uptake and is expressed in the S1 segment of the proximal tubule of the kidneys[11,12].

In addition to the reabsorption of approximately 99% of glucose, recent studies show the kidney takes up lactate, glutamine, glycerol, and alanine and converts them to glucose by the process of gluconephrogenesis, which can account for about 20% of all glucose released into the circulation and nearly 90% of the glucose released by the kidney[13].

The SGLT-2 inhibitors inhibit SGLT-2, which increases renal excretion of glucose thus reducing glucose in the plasma. Due to the minimal glucose uptake by SGLT-1 and the important roles of SGLT-2 in glucose reabsorption, several researchers and manufacturers have turned their attention to SGLT-2 inhibitors for treating hyperglycemia[14-16]. There are several SGLT-2 inhibitors in varying phases of studies including dapagliflozin, empagliflozin, ipragliflozin, ertugliflozin, luseogliflozin, tofogliflozin and LX4211[6,17].

The FDA approved canagliflozin (Invokana®) to treat type 2 diabetes based on the agreement that post marketing studies will be completed for evaluating cardiovascular outcomes, malignancies, severe pancreatitis, hypersensitivity and photosensitivity reactions; liver abnormalities, adverse events during pregnancy, bone safety, and two pediatric studies under the Pediatric Research Equity Act CR[18].

Dapagliflozin was approved in Europe, Australia, Brazil, Mexico and New Zealand as Forxiga®, but the FDA initially delayed its approval as there were concerns of increased breast and bladder cancer in patients taking the drug compared to placebo[19].

In January 2014, the FDA approved dapagliflozin as Farxiga® with six postmarketing studies including a

Table 1 Emerging classes of medications and approaches[8]

SGLT inhibitors
11β-HSD-1 inhibitors
GKA
AMPK agonists
SIRT activators
PTP-1B inhibitors
GCGR antagonists
GR antagonists
Novel insulin sensitizers
GPR119 agonists
Other drugs augmenting GLP-1 secretion: GPR40, G-protein coupled bile acid receptor (TGR5) agonists
Acyl-CoA: DGAT1 inhibitors
FGF-21-receptor agonists
Ranolazine
Other glucometabolic approaches
Other metabolic approaches
Anti-inflammatory approaches
Induction of immune tolerance
Pancreatic beta cell protection and regeneration
Pancreatic islet cell transplantation
Various antidiabetic approaches

SGLT: Sodium-dependent glucose co-transporter; 11beta-HSD-1: 11beta-hydroxysteroid dehydrogenase type 1; GKA: Glucokinase activators; AMPK: Adenosine monophosphate activated protein kinase; SIRT: Sirtuin; PTP-1B: Protein tyrosine phosphatase-1B; GCGR: Glucagon receptor; GR: Glucocorticoid receptor; GPR119: G-protein coupled receptor 119; GLP-1: Glucagon like peptide-1; Acyl-CoA: Acyl-coenzymeA; DGAT1: Diacylglycerol acyltransferase1; FGF-21: Fibroblast growth factor-21.

cardiovascular outcomes trial (CVOT) to evaluate the cardiovascular risk in patients with high cardiovascular disease risk and the evaluation of bladder cancer risk in patients enrolled in the CVOT[20].

Although there are several SGLT-2 inhibitors in varying phases of development, canagliflozin and dapagliflozin will be presented here due to availability of human safety and efficacy data.

Canagliflozin (invokana®) clinical trials

Wilding *et al*[14] designed a randomized, double-blind, placebo-controlled, phase 3, multicenter, 52-wk study to evaluate the safety and efficacy of canagliflozin added to metformin plus sulphonylurea in patients with type 2 diabetes.

The trial, called CANagliflozin Treatment And Trial Analysis-Metformin plus SUIphonylurea, included patients if they were 18-80 years with type 2 diabetes, who were stable on maximum or near maximum dosages of metformin and sulfonylureas with an A1c $\geqslant$ 7% and $\leqslant$ 10.5%[14].

The primary efficacy endpoint was A1c change from baseline to 26 wk. The secondary end points included change in baseline A1c at 52-wk, change in baseline in fasting plasma glucose (FPG), systolic blood pressure (BP), percent change in body weight, triglycerides, and high density lipoprotein (HDL) cholesterol, and percent patients reaching A1c 7%[14]. The investigators evaluated safety by observing adverse event reports, vital signs and laboratory tests[14]. Patients were randomized to receive either 100 mg or 300 mg canagliflozin or placebo in addition to their metformin and sulphonylurea therapies[14].

Results of the study show that 381 (81%) of 469 patients, who were randomized to the study, completed the 52-wk study. By week 26, the A1c was significantly reduced in the canagliflozin 100 mg and 300 mg study arm to -0.85% and 1.06% which was statistically significant compared to baseline and the A1c was sustained over the entire 52 wk study period[14]. Results are presented in Table 2[14]. FPG was significantly improved at 26 wk and 52 wk with both canagliflozin 100 mg and 300 mg compared to placebo. Canagliflozin significantly reduced weight but there were no significant changes with systolic blood pressure, pulse or cholesterol parameters[14].

Safety profile and adverse events: Although investigators reported that adverse effects were higher with canagliflozin than placebo, they were comparable across the treatment groups. Patients on canagliflozin had higher rates of genital mycotic infections compared to placebo, which were described as mild to moderate in severity[14]. Patients who developed a mycotic infection, especially women, had a prior history of genital mycotic infections compared to those women who received canagliflozin and did not have adverse effects[14]. Genital mycotic infections were treated without interrupting canagliflozin therapy[14].

Canagliflozin compared to sitagliptin

Canagliflozin has been shown to be non-inferior to sitagliptin and in another analysis superior to sitagliptin with regard to lowering of A1c[16].

In a randomized, double-blind, active-control, multicenter, phase three, 52-wk study, Schernthaner evaluated the efficacy and safety of canagliflozin 300 mg compared with sitagliptin 100 mg as add-on therapy in patients with type 2 diabetes mellitus inadequately controlled with metformin and a sulfonylurea[16].

The inclusion criteria were similar to the previously described study, and patients were randomized to receive either 300 mg canagliflozin or 100 mg sitagliptin[16]. The primary efficacy endpoint was A1c change from baseline to 52 wk while the secondary endpoints were similar to the previously described study[16].

Results of the study show that 464 (61%) of 755 patients, who were randomized to receive either canagliflozin 300 mg or sitagliptin 100 mg daily, completed the study. Most of the withdrawals were observed in the sitagliptin therapy arm of the trial due to the lack of glycemic rescue therapy[16]. Canagliflozin demonstrated both noninferiority and in another analysis, showed superiority to sitagliptin 100 mg in reducing A1c (-1.03% and -0.66%, respectively). There were greater reductions with canagliflozin *vs* sitagliptin in FPG, body weight, and systolic BP. More patients on canagliflozin compared with sitagliptin achieved A1c < 7.0%, and A1c < 6.5% at week 52, though the authors did not confirm statistical significance[16]. Results are presented in Table 3[16].

Table 2 Results of phase 3, CANagliflozin treatment and trial analysis-metformin plus SUIphonylurea, *n* = 469[14]

Parameters	Canagliflozin 100 mg	Canagliflozin 300 mg	Placebo	Comments
A1c (%) week 26	-0.85	-1.06	-0.13	$P < 0.001$
A1c (%) week 52	-0.74	-0.96	-0.01	$P < 0.001$
% Patients with A1c < 7% week 26	43.2	56.6	18.0	$P < 0.001$
% Patients with A1c < 7% week 52	39.4	52.6	18.7	$P < 0.001$
FPG (mg/dL) week 26	-21.6	-34.2	-	$P < 0.001$
FPG (mg/dL) week 52	-28.8	-37.8	-	$P < 0.001$
Weight	-1.10	-1.7	-	$P < 0.001$
Change in systolic blood pressure (mmHg)	-2.20	-1.6	-	Non significant
Change in pulse (beats/min)	0.90	-1.2	-0.4	Non significant

A1c: Hemoglobin A1c; FPG: Fasting plasma glucose.

Table 3 Results of canagliflozin compared with sitagliptin for patients with type 2 diabetes: (*n* = 755)[16]

Parameters	Canagliflozin 300 mg	Sitagliptin 100 mg	Comments
A1c (%) week 52	-1.03	-0.66	Non inferiority to sitagliptin (upper limit of the 95%CI < 0.3%) and superiority to sitagliptin (upper limit of the 95%CI < 0.0%)
Percent (%) of patients with A1c < 7% at week 52	47.6	35.3	Not significant
Percent (%) of patients with A1c < 6.5% at week 52	22.5	18.9	Not significant
FPG (mg/dL) week 26	-29.9	-5.9	$P < 0.001$
Weight (kg)	-2.3	-0.1	$P < 0.001$
Change in systolic blood pressure (mmHg)	-5.1	0.9	$P < 0.001$
Change in diastolic blood pressure (mmHg)	-3.0	-0.3	Not significant

A1c: Hemoglobin A1c; FPG: Fasting plasma glucose.

Safety profile and adverse events: There were no differences in adverse effects, hypoglycemia or discontinuation of therapy between treatment groups. Nevertheless, canagliflozin had higher rates of genital mycotic infections (vulvovaginitis in females and balanitis in males) compared to sitagliptin[16]. In other studies, canagliflozin is implicated in urinary tract infections, hypoglycemia and gastrointestinal upset when used alone or in combination with other antihyperglycemic therapy[21].

Canagliflozin was associated with a dose dependent increase in serum creatinine, decrease in estimated glomerular filtration rate, renal impairment, and acute failure in patients especially those with moderate renal impairment and hypovolemia[22].

Canagliflozin 100-300 mg is recommended for patients with creatinine clearance > 60 mL/min per 1.73 m^2 and canagliflozin 100-mg is recommended for patients with creatinine clearance of 45-60 mL/min per 1.73m^2[22]. Canagliflozin is not recommended in patients with creatinine clearance of 30-44 mL/min per 1.73 m^2, and it is contraindicated in patients with creatinine clearance of < 30 mL/min per 1.73m^2[22]. Clinicians should assess patients' renal functions when initiating therapy and for long term drug monitoring. This agent will be a safe and efficacious addition to a dual therapy regimen such as metformin and sulfonylurea based on this study[16].

DAPAGLIFLOZIN AS MONOTHERAPY

List *et al*[23] designed a prospective, dose ranging 12-wk, randomized parallel-group, double-blind, placebo-controlled study to evaluate the safety and efficacy of dapagliflozin. The primary objective was to compare the mean change from baseline A1c in type 2, treatment-naïve adult patients (age 18-79) with A1c ≥ 7% and ≤ 10%[23].

Patients were randomly assigned to one of five once-daily dapagliflozin doses (2.5, 5, 10, 20 or 50 mg), metformin XR (750 mg force titration to 1500 mg) or placebo. Investigators also evaluated changes in FPG, weight, and adverse effects[23].

Results of the study show that 348 (89%) of 389, who were randomized to the study completed the study at week 12[23]. At the end of the study, dapagliflozin had statistically significant mean dose-dependent reduction of A1c from -0.55% to -0.90% when compared with placebo -0.18% but not with metformin of -0.73%[23]. Dapagliflozin also had significant reduction in FPG of -16 to -31 mg/dL compared to 6 mg/dL with placebo and -18 mg/dL with metformin[23]. Dapagliflozin caused a weight loss change of -1.3 to 2 kg[23]. In this trial, dapagliflozin did not demonstrate any renal function changes[23]. The percentage of patients achieving A1c < 7% was 40%-59% for the dapagliflozin group *vs* 32% for placebo and 54% for metformin[23]. Hypoglycemia was reported in 6%-10% of patients treated with dapagliflozin but this was not dose related, compared to 4% of placebo patients and 9% of metformin-treated patients[23].

Dapagliflozin in combination with metformin

Henry *et al*[24] conducted two randomized, double-blind,

Table 4 Dapagliflozin in combination with metformin[24]

Parameters	Study 1			Study 2		
	DAPA 5 ± MET	DAPA 5 ± PBO	MET ± PBO	DAPA 10 ± MET	DAPA 10 ± PBO	MET ± PBO
A1c at 24 wk (%)						
Baseline (*n*)	9.21 (185)	9.14 (196)	9.14 (195)	9.1 (202)	9.03 (216)	9.03 (203)
A1c (%) at 24 wk (baseline change)	7.13 (-2.05)	7.96 (-1.19)	7.79 (-1.35)	7.1 (-1.98)	7.59 (-1.45)	7.6 (-1.44)
DAPA ± MET *vs* DAPA	-0.86 (-1.11, -0.62)			-0.53 (-0.74, -0.32)		
P value	< 0.0001			< 0.0001		
DAPA ± MET *vs* MET	-0.70 (-0.94, -0.45)			-0.43 (-0.75, -0.33)		
P value	< 0.0001			< 0.0001		
Patients with A1c < 7% at 24 wk						
n (%)	96/185 (52.4%)	46/196 (22.5%)	68/195 (34.6%)	92/202 (46.6%)	69/216 (31.7%)	72/203 (35.2%)
DAPA ± MET *vs* DAPA	29.9	22.5		14.9		
P value	< 0.0001			0.0012		
DAPA ± MET *vs* MET	17.8			11.3		
P value	< 0.0001			0.0165		
Plasma glucose at 24 wk (mg/dL)						
Baseline FPG (mg/dL)	193.14 (*n* = 192)	190.62 (*n* = 203)	196.56 (*n* = 200)	189.36 (*n* = 209)	197.28 (*n* = 216)	189.72 (*n* = 207)
FPG after 24 wk (baseline change)	132.3 (-61.02)	150.3 (-41.94)	161.1 (-33.48)	130.86 (-60.3)	147.6 (-46.44)	156.42 (-34.74)
DAPA ± MET *vs* DAPA	-19.08			-13.86		
P value	< 0.0001			< 0.0001		
DAPA ± MET *vs* MET	-27.54			-25.56		
P value	< 0.0001			< 0.0001		
Total body weight at 24 wk (kg)						
Baseline weight (*n*)	84.24 (192)	86.20 (203)	85.75 (200)	88.56 (209)	88.53 (219)	87.24 (208)
Change from baseline	-2.66 (-3.14, -2.19)	-2.61 (-3.07, -2.15)	-1.29 (-1.76, -0.82)	-3.33 (-3.80, -2.86)	-2.73 (-3.19, -2.27)	-1.36 (-1.83, -0.89)

DAPA: Dapagliflozin; MET: Metformin; PBO: Placebo; FPG: Fasting plasma glucose; A1c: Hemoglobin A1c.

three-arm 24-wk trials to compare the combination of dapagliflozin plus metformin *vs* dapagliflozin monotherapy and metformin monotherapy to determine if the combination would be an advantage for treatment naïve type 2 diabetes patients with high baseline A1c.

Study 1 compared dapagliflozin 5 mg in combination with metformin XR, dapagliflozin 5 mg in combination with placebo, and metformin XR plus placebo. Study 2 compared dapagliflozin 10 mg in combination with metformin XR, dapagliflozin 10 mg in combination with placebo, and metformin XR plus placebo[24].

Eligible patients had a baseline A1c 7.5%-12%, and the primary endpoint was a change in A1c from baseline while the investigators also evaluated the change in FPG and weight as secondary endpoints[24].

Results show that in both trials, the combination of dapagliflozin and metformin resulted in significantly lower reductions in A1c compared with either metformin or dapagliflozin monotherapy[24]. Results of the study are presented in Table 4[24]. The combination therapy was statistically superior to monotherapy in reduction of FPG and was more effective than metformin for weight reduction. Dapagliflozin 10 mg was non-inferior to metformin in reducing A1c in study 2[24].

Safety profile and adverse events: Adverse effects of mild to moderate cases of genital infection of vulvovaginitis and balanitis and urinary tract infections were reported and treated without discontinuing the study[24]. There were no major hypoglycemic events reported. Diarrhea was more common in patients on combination therapy with metformin than with dapagliflozin therapy alone[24].

Summary of SGLT-2 inhibitors: Canagliflozin and dapagliflozin have been shown to lower renal threshold for glucose in a dose dependent fashion by increasing urinary glucose excretion through SGLT-2 inhibition, which leads to clinical significant reduction in A1c, FPG, and body weight[14,24]. The reduction in renal threshold is above the threshold for hypoglycemia demonstrating this agent has a low risk of hypoglycemia[17]. The SGLT-2 inhibitors can be used with any other agent whether in a treatment naïve patient or a patient with a long history of type 2 diabetes[22,23,25]. Both therapies are safe and tolerable, but clinicians need to observe for genital infections, which can be easily treated without discontinuation of therapy.

METABOLIC APPROACHES TO THERAPY

11β-HSD-1 inhibitors

High levels of glucocorticoids have been associated with hyperglycemia, insulin resistance, dyslipidemia and visceral obesity[4]. 11β-HSD is an enzyme, presenting as two distinct isoenzymes: 11β-HSD-1 and 11β-HSD-2. 11β-HSD-1 is found in the liver and adipose tissue and converts inactive cortisone to active cortisol while 11β-HSD-2 is found primarily in the kidneys and colon and it inactivates glucocorticoids by converting active cortisol to inactive cortisone[4,26].

It has been suggested that the increased glucocorticoid activity in the white adipose tissue by 11β-HSD-1 is a key player in the development of visceral obesity, insu-

Table 5 Efficacy assessment of INCB13739 in combination with metformin[30]

	Placebo	5 mg	15 mg	50 mg	100 mg	200 mg
Baseline A1c (%)	8.3 ± 1	8.2 ± 1	8.3 ± 1	8.3 ± 1	8.2 ± 1	8.2 ± 1
LS mean change A1c (%) from baseline	0.09 ± 1	-0.21 ± 1[b,e]	-0.11 ± 1	-0.09 ± 2	-0.38 ± 1[a,e]	0.47 ± 1[d,h]
A1c > 8% (*n*)	-0.10 ± 0.2 (23)	-0.39 ± 0.2[e] (23)	-0.24 ± 0.2 (18)	-0.65 ± 0.3[b,e] (11)	-0.72 ± 0.2[a,e] (16)	0.65 ± 0.2 (19)
A1c (%) for BMI > 30 mg/m^2 (*n*)	0.17 ± 0.1 (29)	-0.24 ± 0.2[b,f] (23)	-0.10 ± 0.2 (26)	-0.25 ± 0.2[b] (18)	-0.36 ± 0.2[a] (26)	-0.76 ± 0.2[d,h] (18)
Baseline FPG (mg/dL)	179 ± 51	172 ± 41	175 ± 44	178 ± 53	170 ± 64	165 ± 41
LS mean change from baseline (mg/dL)	12.6 ± 6.1	6 ± 6.3	2.3 ± 6.4	-4.7 ± 7.2[b]	-1.6 ± 6.1[b]	-11.5 ± 6.2[d,f]
Weight (kg)	-0.2 ± 0.3	-0.5 ± 0.38	-0.6 ± 0.4[e]	0 ± 0.4	-1.1 ± 0.3[b,e]	-0.9 ± 0.3[h]
HOMA-IR	0.25 ± 0.4	-0.29 ± 0.4	0.33 ± 0.4	-0.42 ± 0.5	0.51 ± 0.4	-1.06 ± 0.4[a,e]

Data are placebo adjusted least-squares (LS) mean change from baseline: mean ± SE. [a]$P < 0.05$, [b]$P < 0.01$, [d]$P < 0.01$, active *vs* Placebo, [e]$P < 0.05$, [f]$P < 0.01$, [h]$P < 0.01$, week 12 *vs* baseline. A1c: Hemoglobin A1c; FPG: Fasting plasma glucose; LS: Least squares; BMI: Body mass index; HOMA-IR: Homeostatic model assessment-insulin resistance.

lin resistance, diabetes, type 2 diabetes, dyslipidemia and hypertension in mice[27]. Increased levels of 11β-HSD-1 in adipose tissue produce a metabolic syndrome in mice while 11β-HSD-1 deficiency or inhibition has beneficial metabolic effects on liver metabolism[27].

In humans, researchers discover that though patients with glucocorticoid excess develop central obesity, yet the circulating glucocorticoid levels are normal. The metabolic syndrome resembles Cushing's syndrome, but without the elevated circulating glucocorticoid levels. Researchers suggest that it is the increased activity of 11β-HSD-1 in humans, which is metabolizing cortisol from cortisone within adipose tissue that may play a major role in the pathophysiology of obesity[28]. Inhibition of this enzyme may potentially decrease weight and blood glucose.

Non selective 11β-HSD-1 inhibitors

Older non-selective 11β-HSD-1 inhibitors such as liquorice and its active metabolite glycyrrhizic and glycyrrhetinic acids inhibit both 11β-HSD-1 and 11β-HSD-2 enzymes[29].

Ingesting liquorice and glycyrrhizic or glycyrrhetinic acids have been shown to produce a type of "mineralocorticoid excess" syndrome, hypertension encephalopathy, and hypokalemic paralysis[29]. It can also cause weight loss, sodium retention, potassium loss, and hypertension through the inhibition of 11β-HSD-2[29].

Carbenoxolone, a non-selective 11β-HSD-1 inhibitor and product of liquorice reduces glucose concentrations and increases weight loss; inhibits hepatic triglyceride production, inhibits lipolysis, and increase HDL-C levels, but also causes sodium retention, potassium loss, and hypertension by inhibiting 11β-HSD-2[29].

Vitamin A enriched diets also decrease fat and improve insulin sensitivity in animals and humans as it may inhibit 11β-HSD-1 and mRNA[29]. These non-selective agents were evaluated in small trials with short durations[29].

Several 11β-HSD-1 inhibitors have been developed and are being tested for patients with obesity and diabetes, including INCB013739, MK0916, PF915275, AMG221 produced by a variety of manufacturers. Results from INCB013739 clinical studies show that 11β-HSD-1 inhibitors when administered to patients with type 2 diabetes for 2 wk prevented the conversion of oral cortisone to cortisol, decreased hepatic gluconeogenesis, decreased fasting plasma glucose and low density lipoprotein cholesterol[30].

Clinical trial of INCB13739 (a 11β-HSD-1 inhibitor)

Rosenstock *et al*[30] evaluated the efficacy and safety of the agent INCB13739 (an 11β-HSD-1 inhibitor) for patients with type 2 diabetes, who were inadequately controlled on a mean dosage of 1.5 g daily of metformin therapy.

The study was a double-blind, placebo-controlled parallel study conducted with 302 type 2 diabetes mellitus patients on metformin therapy with an A1c of 7% to 11%[30]. Patients received one of five dosages (5, 15, 50, 100 or 200 mg) of INCB13739 or placebo once daily for 12 wk in addition to metformin. The primary end point was a change in A1c at the end of 12 wk. Investigators also reviewed FPG, lipids, weight loss, and adverse events[16,30]. Patients had a mean duration of type 2 diabetes of 6.2 years with baseline body mass index of 32.4 kg/m^2, A1c 8.3% and FPG 173 mg/dL[30].

Results of the study show that 228 of 302 (75%) patients completed the study[30]. At the end of the study, INCB13739 resulted in a dose dependent reduction in A1c of -0.38% and -0.47% in the 100 mg and 200 mg groups respectively[30]. However, it was noted that there were more significant A1c changes in obese patients on the higher dosages[30]. In addition, those with A1c > 8% had more significant decrease in A1c which was dosage dependent[30]. Results of the study are presented in Table 5[30]. The investigators reported that at the end of 12 wk, 25% of patients who were randomized to the 100 mg and 200 mg therapy groups achieved an A1c < 7% compared to 9.5% of placebo patients[30]. FPG decreased in a dose and time dependent fashion in the 100-200 mg treatment groups while there was significant weight loss in the 15, 100 and 200 mg groups[30]. The investigators reported that this study group had generally controlled blood pressure and plasma lipids at baseline but there was a modest dose dependent decrease in total cholesterol -7 mg/dL ($P_{trend} = 0.026$) from baseline in the 200 mg group[30]. There was no significant difference with HDL cholesterol[30].

Safety profile and adverse events: The therapy was well

tolerated and adverse events were similar across all treatment groups[30]. There were no serious events reported except for cardiac arrest unrelated to study therapy and there were no hypoglycemia reported. The most common adverse event in four patients was nausea in the 200 mg group but this resolved during continuation of therapy[30].

It was noted that there was also a dose dependent statistically significant reduction in Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) suggesting an insulin sensitizing mechanism of action in the 200 mg group[30]. The authors concluded that in patients with type 2 diabetes inadequately controlled with metformin, INCB13739 added to metformin significantly improved A1C, FPG and HOMA-IR[30]. INCB13739 also decreased weight though it did not affect the waist to hip ratio[30].

Summary: 11β-HSD-1 is increased in the adipose tissues of obese patients and those with the metabolic syndrome. 11β-HSD-1 inhibitors may be a viable option for these patients since it converts inactive cortisol to active cortisol in target tissues, which inhibits pancreatic beta cell insulin production, and prevents peripheral glucose uptake promoting weight loss, and decrease in blood glucose[30]. Researchers and clinicians have questions with regard to effects on the immune system, duration and timing of therapy, the long term effects of weight and lipids, glycemic control, insulin action, atherosclerotic plaque formation and cardiovascular risk[30]. The reduction in A1c was moderate but further studies will answer many of these questions to determine the safety and efficacy of 11β-HSD-1 inhibitors.

Glycogen phosphorylase inhibitors

The liver contributes to glucose production by both gluconeogenesis (glucose synthesis) and glycogenolysis (glycogen breakdown)[31]. Type 2 diabetes is characterized by excessive glucose production and inadequate suppression of hepatic gluconeogenesis postprandially[31].

Except for metformin, the production of gluconeogenesis inhibitors has yielded disappointing results with an increase in compensatory hepatic glycogenolysis, which maintains excessive hepatic glucose production[31,32]. Researchers hypothesized that glycogenolysis inhibition can improve blood glucose control by observing patients with hepatic glycogen storage disease experience intermittent hypoglycemia[31]. Glycogen phosphorylase is an enzyme that catalyzes the breakdown of glycogen to glucose-1-phosphate in the liver and tissues that demand high energy[33].

Hepatic glycogenolysis has a major role in the regulation of plasma glucose levels in diabetic mice, and suggests that glycogen phosphorylase inhibitors may be useful in the treatment of type 2 diabetes[31]. Further studies will elucidate if this is so.

Two types of glycogen phosphorylase inhibitors exist[31]. One is a glucose analog, which binds near the active site of the enzyme, and the other is caffeine and other heteroaromatic analogs which bind at the purine inhibitory site (I-site). The I-site is a target for therapy as compounds which bind at this inhibitory site are more potent in the presence of high glucose concentrations[31]. Researchers hypothesized that the inhibitory activity can be regulated by blood glucose concentrations and the inhibitory activity can decrease as normal blood glucose is achieved, which would decrease the risk of hypoglycemia[31].

CP-91149-a glycogen phosphorylase inhibitor in animal studies: CP-91149 was identified as a potent inhibitor of hepatic glucose production in *in vivo* studies in diabetic ob/ob mice[31]. CP-91149 exhibited rapid dose dependent decreases in plasma glucose concentrations (36-120 mg/dL) at 10, 25, and 50 mg/kg doses ($P < 0.001$) without producing hypoglycemia. Hypoglycemia was defined as glucose < 60 mg/dL for CP91149 in this study[31]. Administration of CP-91149 to normoglycemia non diabetic mice at 25-100 mg/dL did not affect glucose lowering. The glucose lowering of CP91149 was accompanied by an inhibition of hepatic glycogen breakdown in the diabetic ob/ob mice[31].

CP-316819-a glycogen phosphorylase inhibitor: CP-316819 is an analogue of CP-91149, which binds to the inhibitor site of glycogen phosphorylase to prevent its transformation to a more active form of the enzyme[33].

One of the concerns was that this analogue does not demonstrate hepatic specificity, so potentially affecting skeletal tissues and having possible deleterious effects to patients who exercise[33]. In a study by Baker, CP-316819 reduced glycogen phosphorylation activation in rat skeletal muscle at rest and maximal contraction, which produced a modest reduction in muscle lactate production[33]. According to the researcher, the study demonstrated that the concern related to potential negative effects of glycogen phosphorylase inhibition on quality of life due to impaired muscle function are unfounded[33].

Summary of glycogen phosphorylase inhibitors

These findings support the possible use of the glycogen phosphorylase inhibitors as a possible addition to the treatment of patients with type 2 diabetes. Further studies are needed to evaluate the effects of glycogen phosphorylase inhibition after chronic oral dosages and under a variety of exercise activities[33].

PROTEIN TYROSINE PHOSPHATASE 1B INHIBITORS

Type 2 diabetes and obesity are both characterized by insulin and leptin resistance[34,35].

Insulin resistance is found in tissues important for glucose homeostasis such as the liver, fat, central nervous system and muscle[34]. Leptin suppresses food intake and increases energy expenditure, but its levels are elevated in obesity demonstrating leptin resistance. Protein tyrosine phosphatases play a major role in leptin resistance by suppressing leptin signaling[36].

Protein tyrosine phosphatase 1B (PTP-1B) is an enzyme that removes phosphate from tyrosine residues in protein such as insulin receptors, so it is described as a negative regulator for insulin and leptin, by dephosphorylating phosphorylated tyrosine residues from the insulin receptor[34]. PTP-1B activity is increased in insulin resistance and obese patients[34].

Summary

Diabetes mice treat with specific PTP-1B inhibitors exhibited normalized BG control, improved insulin sensitivity, and modulated fat storage, and lipogenesis in adipose tissue[34]. Therefore these inhibitors have emerged as a potential oral agent that can provide a strategy for the treatment of type 2 diabetes and obesity and may work best in patients with beta cell function that releases insulin[35].

Further studies will elucidate if these agents can also be a potential addition to the armamentarium of oral diabetes agents affecting both obesity and the metabolic syndrome.

G-PROTEIN-COUPLED RECEPTOR 119 AGONISTS

A dysfunction in pancreatic β cell leading to decreased insulin secretion is a major abnormality in type 2 diabetes mellitus[37]. The pharmacotherapy approach of stimulating insulin release in a glucose-dependent manner using G-protein-coupled receptor has been investigated[38]. Specifically, G-protein-coupled receptor 119 (GPR119) is largely distributed in pancreatic islet cells, somewhat in the gastrointestinal tract, and found to be involved in glucose metabolism[39-41].

GPR119 may be stimulated by endogenous ligands or synthetic compounds resulting in an elevated cyclic adenosine monophosphate[42]. Studies have shown that stimulation of GPR119 yields glucose-dependent insulin release from the pancreatic β cells, glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide secretions from intestinal cells[42]. Thus, pharmacological agents that target GPR119 results in glucose reduction with low hypoglycemia risk, body weight loss, and potential for pancreatic β cell preservation[42]. These characteristics are very similar to the commercially available GLP-1 agonists, however the studied GPR119 agents may be orally administered. Several GPR119 molecules (GSK1292263, MBX-2982, PSN-821, AR231453, AR-7947) have been studied in preclinical and/or early clinical trials with poor outcomes due to loss of pharmacological effect or minimal glycemic lowering effect[42]. Furthermore, GPR119 agonists have also been considered in combination with DPP-4 inhibitors in an attempt to enhance the GLP-1 effects[42].

Summary

GPR119 agonists have strong potential to meet the needs of patients with type 2 diabetes because of their relative safety profile, lack of weight gain, oral formulation, and possible β cell preservation effect. However, there have been challenges to their development due to potential tachyphylaxis and low anti-hyperglycemia efficacy.

GK ACTIVATORS

GK is a key enzyme in the hexokinase family that facilitates glucose homeostasis *via* glucose phosphorylation and metabolism mainly in the pancreatic β cells and hepatocytes[43-45]. GK functions as a glucose sensor in pancreatic β cells, thereby stimulating glucose-stimulated insulin secretion and regulating glucose metabolism within the liver, including gluconeogenesis, glycolysis, glycogen synthesis, glucose oxidation, lipogenesis, urea, and uric acid production[43,45-48].

Since the initial development of small molecules known as GK activators (GKAs) that bind to an allosteric site of the enzyme in 2003, more than 150 patents have been established[49-51]. Preclinical and clinical phase trials of GKAs have demonstrated glucose lowering effect in both animal and humans[52]. This novel class of anti-diabetic agents holds promise particularly because both mechanistic actions of GK are impaired in type 2 diabetes[53]. However, there are concerns about potential side effects including hyperlipidemia, hypoglycemia, and fatty liver that may limit the development of GKAs[54]. For example, a small Phase I clinical trial involving the GKA piragliatin was discontinued in type 2 diabetes patients with unrevealed rationale[55].

Another GKA molecule, MK0941 was evaluated in a 54-wk Phase Ⅱ trial in type 2 diabetes patients, but was discontinued because of observed hyperlipidemia, vascular hypertension and early therapy failure[56].

Summary of GKA

GKAs offer a unique pharmacotherapeutics approach to type 2 diabetes management and have demonstrated useful potential in glycemic management. However, further development is needed to address the potential side effects observed in clinical trials. Additional advancements may include modifications of the GKAs structures and activities to minimize hypoglycemia, hyperlipidemia, fatty liver, and vascular hypertension[44].

CONCLUSION

The management of type 2 diabetes present many treatment challenges, but new and emerging drug therapies are a welcome addition to complement the current agents. The SGLT-2 inhibitors have shown significant benefits as monotherapy and in combination with available agents like metformin, sulphonylurea and insulin therapy. The selective 11β-HSD-1 inhibitor is another class of possibly safe and efficacious agent that lowers fasting blood glucose, A1c and weight, although the A1c lowering was modest. The glycogen phosphorylase inhibitors appear to show rapid and safe blood glucose decreases in mice without the risk of hypoglycemia. Hope-

fully similar results translate into human studies. PTP-1B is still in clinical trials and may show significant decrease in weight and glucose levels in insulin and leptin resistant patients. Mice studies show positive results of normalized blood glucose control, improved insulin sensitivity and improvements in lipogenesis. The GPR119 agonists have strong potential for meeting the needs of type 2 diabetes patients because of their safety profile, lack of weight gain and possible beta cell preservation effect. However, the GK inhibitors may have some potential problems as agents so far have been discontinued due to dyslipidemia, vascular hypertension and early therapy failure. Prescribers and pharmacists may have to recognize that these new agents may not be first line agents due to costs, monitoring parameters, modest reductions of A1c, and lack of cardiovascular disease data. Further studies will help to more clearly define these new and emerging anti-hyperglycemia agents' roles in therapy.

REFERENCES

1 **Akkati S**, Sam KG, Tungha G. Emergence of promising therapies in diabetes mellitus. *J Clin Pharmacol* 2011; **51**: 796-804 [PMID: 20705952 DOI: 10.1177/0091270010376972]
2 **American Diabetes Association**. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2013; **36**: S67-S74 [DOI: 10.2337/dc13-S067]
3 **International Diabetes Federation**. IDF Diabetes Atlas. 6thed. Brussels, Belgium: International Diabetes Federation, 2013
4 **Centers for Disease Control and Prevention**. National Diabetes Facts Sheet: national estimates and general information on diabetes and prediabetes in the United States, 2011. Available from: URL: http: //www.cdc.gov/diabetes/pubs/pdf/ndfs_2011.pdf
5 **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]
6 **Ahmed I**, Ukrainski M, Bischoff L, Gandrabura T. On the horizon: new oral therapies for type 2 diabetes mellitus. *Inter J Diabetol Vascul Dis Res* 2013; **1**: 301-306
7 **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]
8 **Research and Markets**. Competitor analysis: emerging diabetes drugs. Available from: URL: http: //www.researchandmarkets.com/reports/650944/competitor_analysis_emerging_diabetes_drugs.pdf
9 **DeFronzo RA**, Davidson JA, Del Prato S. The role of the kidneys in glucose homeostasis: a new path towards normalizing glycaemia. *Diabetes Obes Metab* 2012; **14**: 5-14 [PMID: 21955459 DOI: 10.1111/j.1463-1326.2011.01511.x]
10 **Wood IS**, Trayhurn P. Glucose transporters (GLUT and SGLT): expanded families of sugar transport proteins. *Br J Nutr* 2003; **89**: 3-9 [PMID: 12568659 DOI: 10.1079/BJN2002763]
11 **Bakris GL**, Fonseca VA, Sharma K, Wright EM. Renal sodium-glucose transport: role in diabetes mellitus and potential clinical implications. *Kidney Int* 2009; **75**: 1272-1277 [PMID: 19357717 DOI: 10.1038/ki.2009.87]
12 **Nguyen Q**, Thomas K, Lyons K, Nguyen L, Plodkowski R. Current therapies and emerging drugs in the pipeline for type 2 diabetes. *Am Health Drug Benefits* 2011; **4**: 303-311
13 **Gerich JE**, Meyer C, Woerle HJ, Stumvoll M. Renal gluconeogenesis: its importance in human glucose homeostasis. *Diabetes Care* 2001; **24**: 382-391 [PMID: 11213896 DOI: 10.2337/diacare.24.2.382]
14 **Wilding JP**, Charpentier G, Hollander P, González-Gálvez G, Mathieu C, Vercruysse F, Usiskin K, Law G, Black S, Canovatchel W, Meininger G. Efficacy and safety of canagliflozin in patients with type 2 diabetes mellitus inadequately controlled with metformin and sulphonylurea: a randomised trial. *Int J Clin Pract* 2013; **67**: 1267-1282 [PMID: 24118688 DOI: 10.1111/ijcp.12322]
15 **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: 10.1007/s00125-013-3039-1]
16 **Schernthaner G**, Gross JL, Rosenstock J, Guarisco M, Fu M, Yee J, Kawaguchi M, Canovatchel W, Meininger G. Canagliflozin compared with sitagliptin for patients with type 2 diabetes who do not have adequate glycemic control with metformin plus sulfonylurea: a 52-week randomized trial. *Diabetes Care* 2013; **36**: 2508-2515 [PMID: 23564919 DOI: 10.2337/dc12-2491]
17 **Raskin P**. Sodium-glucose cotransporter inhibition: therapeutic potential for the treatment of type 2 diabetes mellitus. *Diabetes Metab Res Rev* 2013; **29**: 347-356 [PMID: 23463735 DOI: 10.1002/dmrr.2403]
18 **FDA: US Food and Drug Administration**. FDA approves Invokana to treat type 2 diabetes: First in a new class of diabetes drugs. Available from: URL: http: //www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm345848.htm
19 **Food and Drug Administration**. Center for Drug Evaluation and Research: Endocrinology and Metabolic Drug Advisory Committee. 2011. Available from: URL: http: //www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/EndocrinologicandMetabolicDrugsAdvisoryCommittee/UCM268727.pdf
20 **Food and Drug Administration: FDA News Release**. FDA approves Farxiga to treat type 2 diabetes. Available from: URL: http: //www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm380829.htm
21 **Riser Taylor S**, Harris KB. The clinical efficacy and safety of sodium glucose cotransporter-2 inhibitors in adults with type 2 diabetes mellitus. *Pharmacotherapy* 2013; **33**: 984-999 [PMID: 23744749 DOI: 10.1002/phar.1303]
22 **Janssen Pharmaceuticals**. HIGHLIGHTS OF PRESCRIBING INFORMATION. Available from: URL: http: //www.invokanahcp.com/prescribing-information.pdf
23 **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]
24 **Henry RR**, Murray AV, Marmolejo MH, Hennicken D, Ptaszynska A, List JF. Dapagliflozin, metformin XR, or both: initial pharmacotherapy for type 2 diabetes, a randomised controlled trial. *Int J Clin Pract* 2012; **66**: 446-456 [PMID: 22413962 DOI: 10.1111/j.1742-1241.2012.02911.x]
25 **Clar C**, Gill JA, Court R, Waugh N. Systematic review of SGLT2 receptor inhibitors in dual or triple therapy in type 2 diabetes. *BMJ Open* 2012; **2**: [PMID: 23087012 DOI: 10.1136/bmjopen-2012-001007]
26 **Gathercole LL**, Lavery GG, Morgan SA, Cooper MS, Sinclair AJ, Tomlinson JW, Stewart PM. 11β-Hydroxysteroid dehy-

drogenase 1: translational and therapeutic aspects. *Endocr Rev* 2013; **34**: 525-555 [PMID: 23612224 DOI: 10.1210/er.2012-1050]
27 **Morton NM**, Paterson JM, Masuzaki H, Holmes MC, Staels B, Fievet C, Walker BR, Flier JS, Mullins JJ, Seckl JR. Novel adipose tissue-mediated resistance to diet-induced visceral obesity in 11 beta-hydroxysteroid dehydrogenase type 1-deficient mice. *Diabetes* 2004; **53**: 931-938 [PMID: 15047607 DOI: 10.2337/diabetes.53.4.931]
28 **Tomlinson JW**, Moore J, Cooper MS, Bujalska I, Shahmanesh M, Burt C, Strain A, Hewison M, Stewart PM. Regulation of expression of 11beta-hydroxysteroid dehydrogenase type 1 in adipose tissue: tissue-specific induction by cytokines. *Endocrinology* 2001; **142**: 1982-1989 [PMID: 11316764]
29 **Anagnostis P**, Katsiki N, Adamidou F, Athyros VG, Karagiannis A, Kita M, Mikhailidis DP. 11beta-Hydroxysteroid dehydrogenase type 1 inhibitors: novel agents for the treatment of metabolic syndrome and obesity-related disorders? *Metabolism* 2013; **62**: 21-33 [PMID: 22652056 DOI: 10.1016/j.metabol.2012.05.002]
30 **Rosenstock J**, Banarer S, Fonseca VA, Inzucchi SE, Sun W, Yao W, Hollis G, Flores R, Levy R, Williams WV, Seckl JR, Huber R. The 11-beta-hydroxysteroid dehydrogenase type 1 inhibitor INCB13739 improves hyperglycemia in patients with type 2 diabetes inadequately controlled by metformin monotherapy. *Diabetes Care* 2010; **33**: 1516-1522 [PMID: 20413513 DOI: 10.2337/dc09-2315]
31 **Martin WH**, Hoover DJ, Armento SJ, Stock IA, McPherson RK, Danley DE, Stevenson RW, Barrett EJ, Treadway JL. Discovery of a human liver glycogen phosphorylase inhibitor that lowers blood glucose in vivo. *Proc Natl Acad Sci USA* 1998; **95**: 1776-1781 [PMID: 9465093 DOI: 10.1073/pnas.95.4.1776]
32 **Somsák L**, Nagya V, Hadady Z, Docsa T, Gergely P. Glucose analog inhibitors of glycogen phosphorylases as potential antidiabetic agents: recent developments. *Curr Pharm Des* 2003; **9**: 1177-1189 [PMID: 12769745 DOI: 10.2174/1381612033454919]
33 **Baker DJ**, Timmons JA, Greenhaff PL. Glycogen phosphorylase inhibition in type 2 diabetes therapy: a systematic evaluation of metabolic and functional effects in rat skeletal muscle. *Diabetes* 2005; **54**: 2453-2459 [PMID: 16046314 DOI: 10.2337/diabetes.54.8.2453]
34 **Johnson TO**, Ermolieff J, Jirousek MR. Protein tyrosine phosphatase 1B inhibitors for diabetes. *Nat Rev Drug Discov* 2002; **1**: 696-709 [PMID: 12209150 DOI: 10.1038/nrd895]
35 **Chen YT**, Tang CL, Ma WP, Gao LX, Wei Y, Zhang W, Li JY, Li J, Nan FJ. Design, synthesis, and biological evaluation of novel 2-ethyl-5-phenylthiazole-4-carboxamide derivatives as protein tyrosine phosphatase 1B inhibitors with improved cellular efficacy. *Eur J Med Chem* 2013; **69**: 399-412 [PMID: 24090912 DOI: 10.1016/j.ejmech.2013.09.017]
36 **St-Pierre J**, Tremblay ML. Modulation of leptin resistance by protein tyrosine phosphatases. *Cell Metab* 2012; **15**: 292-297 [PMID: 22405067 DOI: 10.1016/j.cmet.2012.02.004]
37 **Chu ZL**, Jones RM, He H, Carroll C, Gutierrez V, Lucman A, Moloney M, Gao H, Mondala H, Bagnol D, Unett D, Liang Y, Demarest K, Semple G, Behan DP, Leonard J. A role for beta-cell-expressed G protein-coupled receptor 119 in glycemic control by enhancing glucose-dependent insulin release. *Endocrinology* 2007; **148**: 2601-2609 [PMID: 17289847 DOI: 10.1210/en.2006-1608]
38 **Ha TY**, Kim YS, Kim CH, Choi HS, Yang J, Park SH, Kim DH, Rhee JK. Novel GPR119 agonist HD0471042 attenuated type 2 diabetes mellitus. *Arch Pharm Res* 2014; **37**: 671-678 [PMID: 23897163 DOI: 10.1007/s12272-013-0209-0]
39 **Soga T**, Ohishi T, Matsui T, Saito T, Matsumoto M, Takasaki J, Matsumoto S, Kamohara M, Hiyama H, Yoshida S, Momose K, Ueda Y, Matsushime H, Kobori M, Furuichi K. Lysophosphatidylcholine enhances glucose-dependent insulin secretion via an orphan G-protein-coupled receptor. *Biochem Biophys Res Commun* 2005; **326**: 744-751 [PMID: 15607732 DOI: 10.1016/j.bbrc.2004.11.120]
40 **Bonini J**, Borowsky B, Adham N, inventors. Synaptic Pharmaceutical Corporation, assignee. Methods of identifying compounds that bind to SNORF25 receptors. USA patent 6468756 B1. 2002
41 **Chu ZL**, Carroll C, Alfonso J, Gutierrez V, He H, Lucman A, Pedraza M, Mondala H, Gao H, Bagnol D, Chen R, Jones RM, Behan DP, Leonard J. A role for intestinal endocrine cell-expressed g protein-coupled receptor 119 in glycemic control by enhancing glucagon-like Peptide-1 and glucose-dependent insulinotropic Peptide release. *Endocrinology* 2008; **149**: 2038-2047 [PMID: 18202141 DOI: 10.1210/en.2007-0966]
42 **Kang SU**. GPR119 agonists: a promising approach for T2DM treatment? A SWOT analysis of GPR119. *Drug Discov Today* 2013; **18**: 1309-1315 [PMID: 24060477 DOI: 10.1016/j.drudis.2013.09.011]
43 **Oh YS**, Lee YJ, Park K, Choi HH, Yoo S, Jun HS. Treatment with glucokinase activator, YH-GKA, increases cell proliferation and decreases glucotoxic apoptosis in INS-1 cells. *Eur J Pharm Sci* 2014; **51**: 137-145 [PMID: 24056026 DOI: 10.1016/j.ejps.2013.09.005]
44 **Matschinsky FM**, Zelent B, Doliba N, Li C, Vanderkooi JM, Naji A, Sarabu R, Grimsby J. Glucokinase activators for diabetes therapy: May 2010 status report. *Diabetes Care* 2011; **34** Suppl 2: S236-S243 [PMID: 21525462 DOI: 10.2337/dc11-s236]
45 **Matschinsky FM**, Zelent B, Doliba NM, Kaestner KH, Vanderkooi JM, Grimsby J, Berthel SJ, Sarabu R. Research and development of glucokinase activators for diabetes therapy: theoretical and practical aspects. *Handb Exp Pharmacol* 2011; (**203**): 357-401 [PMID: 21484579 DOI: 10.1007/978-3-642-17214-4_15]
46 **Arden C**, Petrie JL, Tudhope SJ, Al-Oanzi Z, Claydon AJ, Beynon RJ, Towle HC, Agius L. Elevated glucose represses liver glucokinase and induces its regulatory protein to safeguard hepatic phosphate homeostasis. *Diabetes* 2011; **60**: 3110-3120 [PMID: 22013014 DOI: 10.2337/db11-0061]
47 **Agius L**. Glucokinase and molecular aspects of liver glycogen metabolism. *Biochem J* 2008; **414**: 1-18 [PMID: 18651836 DOI: 10.1042/BJ20080595]
48 **Nissim I**, Horyn O, Nissim I, Daikhin Y, Wehrli SL, Yudkoff M, Matschinsky FM. Effects of a glucokinase activator on hepatic intermediary metabolism: study with 13C-isotopomer-based metabolomics. *Biochem J* 2012; **444**: 537-551 [PMID: 22448977 DOI: 10.1042/BJ20120163]
49 **Grimsby J**, Sarabu R, Corbett WL, Haynes NE, Bizzarro FT, Coffey JW, Guertin KR, Hilliard DW, Kester RF, Mahaney PE, Marcus L, Qi L, Spence CL, Tengi J, Magnuson MA, Chu CA, Dvorozniak MT, Matschinsky FM, Grippo JF. Allosteric activators of glucokinase: potential role in diabetes therapy. *Science* 2003; **301**: 370-373 [PMID: 12869762 DOI: 10.1126/science.1084073]
50 **Sarabu R**, Berthel SJ, Kester RF, Tilley JW. Novel glucokinase activators: a patent review (2008 - 2010). *Expert Opin Ther Pat* 2011; **21**: 13-33 [PMID: 21155690 DOI: 10.1517/13543776.2011.542413]
51 **Filipski KJ**, Futatsugi K, Pfefferkorn JA, Stevens BD. Glucokinase activators. *Pharm Pat Anal* 2012; **1**: 301-311 [PMID: 24236843 DOI: 10.4155/ppa.12.26]
52 **Matschinsky FM**. GKAs for diabetes therapy: why no clinically useful drug after two decades of trying? *Trends Pharmacol Sci* 2013; **34**: 90-99 [PMID: 23305809 DOI: 10.1016/j.tips.2012.11.007]
53 **Matschinsky FM**. Assessing the potential of glucokinase activators in diabetes therapy. *Nat Rev Drug Discov* 2009; **8**: 399-416 [PMID: 19373249 DOI: 10.1038/nrd2850]
54 **Gude D**. Red carpeting the newer antidiabetics. *J Pharmacol Pharmacother* 2012; **3**: 127-131 [PMID: 22629086 DOI: 10.4103/0976-500X.95507]
55 **Bonadonna RC**, Heise T, Arbet-Engels C, Kapitza C, Avogaro A, Grimsby J, Zhi J, Grippo JF, Balena R. Piragliatin

(RO4389620), a novel glucokinase activator, lowers plasma glucose both in the postabsorptive state and after a glucose challenge in patients with type 2 diabetes mellitus: a mechanistic study. *J Clin Endocrinol Metab* 2010; **95**: 5028-5036 [PMID: 20739378 DOI: 10.1210/jc.2010-1041]

56 **Meininger GE**, Scott R, Alba M, Shentu Y, Luo E, Amin H, Davies MJ, Kaufman KD, Goldstein BJ. Effects of MK-0941, a novel glucokinase activator, on glycemic control in insulin-treated patients with type 2 diabetes. *Diabetes Care* 2011; **34**: 2560-2566 [PMID: 21994424 DOI: 10.2337/dc11-1200]

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REVIEW

12q24 locus association with type 1 diabetes: *SH2B3* or *ATXN2*?

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Abstract

Genetic linkage analyses, genome-wide association studies of single nucleotide polymorphisms, copy number variation surveys, and mutation screenings found the human chromosomal 12q24 locus, with the genes *SH2B3* and *ATXN2* in its core, to be associated with an exceptionally wide spectrum of disease susceptibilities. Hematopoietic traits of red and white blood cells (like erythrocytosis and myeloproliferative disease), autoimmune disorders (like type 1 diabetes, coeliac disease, juvenile idiopathic arthritis, rheumatoid arthritis, thrombotic antiphospholipid syndrome, lupus erythematosus, multiple sclerosis, hypothyroidism and vitiligo), also vascular pathology (like kidney glomerular filtration rate deficits, serum urate levels, plasma beta-2-microglobulin levels, retinal microcirculation problems, diastolic and systolic blood pressure and hypertension, cardiovascular infarction), furthermore obesity, neurodegenerative conditions (like the polyglutamine-expansion disorder spinocerebellar ataxia type 2, Parkinson's disease, the motor-neuron disease amyotrophic lateral sclerosis, and progressive supranuclear palsy), and finally longevity were reported. Now it is important to clarify, in which ways the loss or gain of function of the locally encoded proteins SH2B3/LNK and ataxin-2, respectively, contribute to these polygenic health problems. SH2B3/LNK is known to repress the JAK2/ABL1 dependent proliferation of white blood cells. Its null mutations in human and mouse are triggers of autoimmune traits and leukemia (acute lymphoblastic leukemia or chronic myeloid leukemia-like), while missense mutations were found in erythrocytosis-1 patients. Ataxin-2 is known to act on RNA-processing and trophic receptor internalization. While its polyglutamine-expansion mediated gain-of-function causes neuronal atrophy in human and mouse, its deletion leads to obesity and insulin resistance in mice. Thus, it is conceivable that the polygenic pathogenesis of type 1 diabetes is enhanced by an SH2B3-dysregulation-mediated predisposition to autoimmune diseases that conspires with an ATXN2-deficiency-mediated predisposition to lipid and glucose metabolism pathology.

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Key words: Diabetes mellitus type 1; 12q24; *ATXN2*; Obesity; *SH2B3*; Autoimmune

Core tip: Within the multifactorial pathogenesis of type 1 diabetes mellitus (T1D), a genetic risk mediated by the chromosome 12q24 locus was consistently observed. Mutations in the *ATXN2* gene there trigger the pathogenesis of obesity, while mutations in the SH2B3 gene there trigger the pathogenesis of autoimmune processes. Given that both genes show co-regulated expression, their combined effects may drive these two core aspects of T1D. Tissue and phenotype studies of mouse mutants will identify molecular targets for causal therapies.

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INTRODUCTION

The pathogenesis of many common multifactorial diseases was successfully elucidated over the past years, principally through genome-wide association studies (GWAS) in many thousands of sporadic patients *vs* control individuals. For diabetes mellitus type 1 (T1D), more than 40 chromosomal loci were uncovered to modulate disease risk[1,2]. However, now the challenge consists in establishing causality between one of the multiple genes contained in any locus and one of the disease features. One promising approach is the careful consideration of phenotypes and pathology caused by disruption or overexpression of any candidate gene, *e.g.*, in mouse, and the subsequent comparison with relevant traits that occur within the first years of the disease course. Thus, clinical information may help to guide the characterization of mutant animals, while conversely the tissue analysis of mutant animals may help to elucidate presymptomatic stages of disease. A particularly complex example is the subject of this review-the association of T1D and many other medical conditions with mostly two single nucleotide polymorphisms (SNPs) on chromosome 12q24-rs3184504 and rs653178.

THE EXCEPTIONALLY PLEIOTROPIC DISEASE SUSCEPTIBILITY LOCUS ON CHROMOSOME 12Q24 EXTENDS FROM THE *SH2B3* GENE ACROSS THE *ATXN2* GENE, BUT MAY STRETCH BEYOND THESE BORDERS

Chromosome 12q contains one of the largest blocks of linkage disequilibrium (LD) in the human genome[3]. It was observed early on in European/Asian/African populations and found to span > 1 Megabase pairs (Mbp) across several genes including the growth repressor *SH2B3*, the RNA processing factor *ATXN2*, the nuclear localization inhibitor *BRAP*, the mitochondrial fatty acid beta-oxidation enzyme *ACAD10*, the alcohol metabolism enzyme *ALDH2*, and the stress kinase *MAPKAPK5*[4]. The core LD block was localized to exon 1 of the *ATXN2* gene in a population of European ancestry, and was explained by positive selection of the (CAG)-repeat size in this exon[4]. Indeed, the most frequently observed disease associations at this 12q24 locus are within a 200000 basepairs (bp) fragment, which comprises the *ATXN2* gene and the immediately adjacent *SH2B3* gene (Figure 1). According to the United States National Center for Biotechnology Information reference sequences, human *SH2B3* is transcribed in orientation from the centromere, covering about 46000 bp, and spans 9 predicted exons to constitute an mRNA of 5425 nucleotides, which encodes a protein of 575 amino acids. *ATXN2* is transcribed in orientation from the telomere, covering about 147000 bp, and spans 24 predicted exons with several splice-isoforms, of which the longest constitutes an mRNA of 4712 nucleotides and encodes a protein of 1313 amino acids. The missense SNP rs3184504 in *SH2B3* open reading frame (resulting in the substitution W262R) was observed in perfect cosegregation ($r^2 = 1$) with the SNP rs653178 deep within intron 2 of the *ATXN2* gene[5], in spite of a physical distance of 123148 bp. Since rs653178 is far away from *ATXN2* splice sites and since the W262 codon in SH2B3 is not conserved between human and mouse[6], both of these polymorphisms are probably innocent bystanders and are noticed only through their frequency, depending on their random distribution within population stratifications. They are presumably coinherited with other rare sequence variants, *e.g.*, within the promoters or within the mRNA 3'-untranslated regions, which alter the transcript expression levels slightly upwards or downwards. Indeed, both of these cosegregating *SH2B3* and *ATXN2* variants correlated with significant changes in the expression of both *ATXN2* and *SH2B3* mRNAs[7]. This coinheritance together with correlated expression changes makes it inherently difficult to establish causality between any of the individual traits within a complex disease and any of the neighbouring genes. This is exemplified by the allocation of six hematologic and three blood pressure traits to the region from *SH2B3* to *ATXN2* by genome-wide studies, reflecting the exceptional pleiotropy of this locus[8]. The 12q24 linkage disequilibrium block in some studies of restricted populations included further genes, namely *CUTL2*, *FAM109A*, *SH2B3*, *ATXN2*, *BRAP*, *ACAD10*, *ALDH2*, *MAPKAPK5*, *TMEM116*, *ERP29*[9], *NAA25/C12orf30*, *TRAFD1*, *HECTD4/C12orf51*, *RPL6*, *PTPN11*[10-12], thus extending across 1.5 Mbp. For these reasons it is crucial to consider monogenic mutants for each gene and their phenotypic effects, so as to decide which of them might contribute to each of the diseases. However, for most of these genes the relevant mouse mutants are not yet characterized.

NULL MUTATIONS IN MOUSE AND HUMAN DEMONSTRATE *SH2B3* TO REPRESS THE PROLIFERATION OF WHITE BLOOD CELLS, IN PARTICULAR B-LYMPHOCYTES

The generation of mice with deletion of SH2B3 (also called Lnk) demonstrated primary splenomegaly and extramedullary hematopoiesis with progenitor hypersensitivity to various cytokines[13]. It caused the accumulation of pre-B and immature B-lymphocytes in enlarged spleens as well as an increase in B-lineage cells in the bone marrow, in parallel to unimpaired T-cell de-

SH2B3-ATXN2 genomic locus

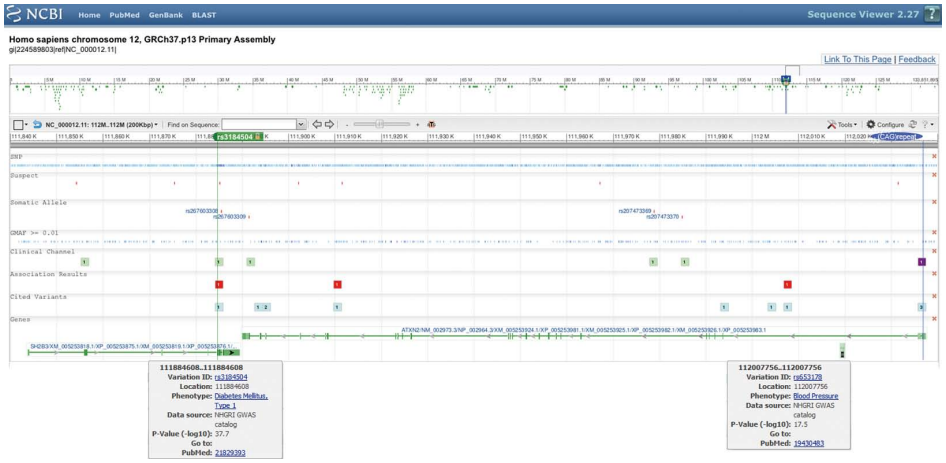

Figure 1 The core 200000 bp region of the chromosome 12q24 locus covering the immediately adjacent *SH2B3* and *ATXN2* genes, with an illustration of the single nucleotide polymorphism rs3184504 encoding the W272R missense variant of the SH2B3/LNK protein (as shown in the United States National Center for Biotechnology Information database) as well as the (CAG)-repeat structure encoding the unstable polyglutamine domain of the ataxin-2 protein.

velopment in thymus[14]. It accelerated and exacerbated oncogenic JAK2-induced myeloproliferative diseases through an expansion of myeloid progenitors, accelerated myelofibrosis and finally features of chronic myeloid leukemia (CML). These murine data supported notions that SH2B3 directly inhibits oncogenic JAK2 and cooperates with the *BCR/ABL* oncogene in the development of CML[15]. Deletion of SH2B3 was also observed in a genomic and transcriptomic study of patients with BCR-ABL1-positive acute lymphoblastic leukemia with poor outcome (Ph-like ALL), together with promising therapeutic benefits from tyrosine kinase inhibitors[16]. Human germline homozygous *SH2B3* mutations including a frameshift with translation stop resulted in growth retardation, high white cell counts in parallel to anemia and thrombocytopenia, splenomegaly and liver cirrhosis, autoimmune Hashimoto thyroiditis, speech delay and ALL. In addition, this study identified homozygous somatic *SH2B3* frameshift mutations in ALL cases[17]. A 5 bp deletion of SH2B3, which was predicted to affect both the PH domain and the SH2 domain, manifested clinically as primary myelofibrosis. In contrast, a somatic *E208Q* missense mutation in the PH domain was observed in a patient with essential thrombocythemia[18]. SH2B3 was also shown to interact with platelet-derived growth factor receptor and repress its downstream signaling[19]. Interestingly, a selective increase in red blood cells (isolated erythrocytosis) was observed in two individuals with the *SH2B3* missense mutations E208X and A215V[20]. However, SH2B3 sequencing in 23 erythrocytosis patients uncovered only one non-synonymous polymorphism of unclear relevance[6]. Systematic SH2B3 sequencing analysis in 42 patients with chronic phase myeloproliferative neoplasms detected a missense mutation in 7% of cases, either in the SH2 domain or in the C-terminal domain, which were always accompanied by a *JAK2* mutation[21]. Myeloproliferative *SH2B3* mutations within the PH domain were also shown to reduce SH2B3 function without altering its binding properties to JAK2, CBL and 14-3-3[22]. An analysis of peripheral mononuclear blood cells stimulated with anti-CD28 and anti-CD3 antibodies detected an increased proliferation of T-lymphocytes in carriers of the W262R missense SH2B3 variant, independent of the presence of juvenile type 1 diabetes[23]. *In vitro* studies had previously shown SH2B3 to attenuate the ability of SH2B1 to promote JAK2 activation and subsequent tyrosine phosphorylation of insulin receptor substrate-1 by JAK2[24]. SH2B3-deficient hematopoietic stem cells displayed an increased postnatal expansion and enhanced thrombopoietin responsiveness[25]. In subsequent studies they showed increased resistance to apoptosis due to enhanced expression of Bcl-xL upon thrombopoietin stimulation[26]. A limitation of growth by SH2B3 was also observed in the rat neuronal PC12 cell line and in primary cortical neurons, where neurotrophin-induced neurite outgrowth was downregulated by the binding of SH2B3 to the phosphorylated neurotrophin receptor TrkA and the repression of downstream signaling[27].

AUTOIMMUNE DISEASES (EOSINOPHIL NUMBERS, COELIAC DISEASE, JUVENILE IDIOPATHIC ARTHRITIS, RHEUMATOID ARTHRITIS, THROMBOTIC ANTIPHOSPHOLIPID SYNDROME, LUPUS ERYTHEMATOSUS, MULTIPLE SCLEROSIS, HYPOTHYROIDISM, VITILIGO) MAY BE MODULATED BY SH2B3

Possibly as an effect of SH2B3 on B-lymphocyte proliferation, the 12q24 locus modulates the risk for various autoimmune diseases. A GWAS in the Icelandic popula-

tion studying eosinophil counts observed association with the *SH2B3* SNP rs3184504[28]. A GWAS into coeliac disease found the *SH2B3* SNP rs3184504 and the *ATXN2* intronic SNP rs653178 to be associated[29]. Follow up studies of coeliac disease focusing on 9 and 11 candidate SNPs confirmed the association with *SH2B3*[30,31], and reported upregulation of *SH2B3* mRNA expression levels in intestinal mucosa to be triggered by coeliac disease and by the risk allele T of the *SH2B3* SNP rs3184504[31]. Further haplotype studies were confirmatory, and functional experiments indicated that carriers of the rs3184504 risk allele show stronger activation of the NOD2 recognition pathway in response to lipopolysaccharides and muramyl dipeptide[32]. A candidate study of sixteen SNPs known from coeliac disease and from T1D found an association of the *ATXN2* SNP rs653178 with juvenile idiopathic arthritis[33]. GWAS studies into rheumatoid arthritis indicated association with *SH2B3* particularly among rheumatoid-factor-positive patients[34]. A GWAS meta-analysis confirmed that the *ATXN2* intronic SNP rs653178 is associated not only with coeliac disease, but also with rheumatoid arthritis[35]. A study of thrombophilia in antiphospholipid antibody positive individuals by array-comparative genomic hybridization analysis of copy number variations with subsequent fine mapping identified a risk haplotype comprising one *SH2B3* SNP and two *ATXN2* SNPs[36]. A GWAS of systemic lupus erythematosus observed association with the SNP rs17696736 within the *ERP29* gene downstream from *SH2B3*[9]. A candidate study of 12 SNPs in almost 3000 Spanish multiple sclerosis patients detected association with the *SH2B3* SNP rs3184504[37]. A GWAS into hypothyroidism reported the *SH2B3* SNP rs3184504 to be associated, with autoimmune Hashimoto thyroiditis as a likely explanation for this observation[38]. A GWAS into the autoimmune skin disease vitiligo reported an association with the 12q24 locus extending from the *SH2B3* across the *ATXN2* gene[39].

T1D MELLITUS

The first GWAS into T1D encountered a maximal association with the 12q24 SNP rs17696736 in an intron of the *C12ORF30/NAA25* gene, while the effect was consistently observed also in its neighbourhood across a 1.5 Mbp LD block[10]. An extended GWAS confirmed this observation and pointed out that the association with the W272R missense variant encoded in exon 3 of SH2B3 was sufficient to model the regional effect[40]. GWAS of additional cases corroborated the association with *SH2B3*[41], a further GWAS with meta-analysis and combined comparisons supported the association with rs3184504[42], and also a GWAS of affected sib-pair families showed association with the region from the *SH2B3* SNP rs739496 across the *ERP29* SNP rs17696736 until the SNP rs10850061 beyond PTPN11[11,43]. GWAS of autoantibody positive T1D patients again detected the association with SH2B3[44,45]. GWAS of soluble intercellular adhesion molecule-1 levels as an endothelium-derived inflammatory biomarker in diabetes and infarction also showed the association with the *SH2B3* SNP rs3184504[46]. Candidate studies of 2 and 21 SNPs in T1D cases from Russia and United States, respectively, replicated the SH2B3 association[47,48]. Since the effect is so consistent, *SH2B3* SNP genotyping was integrated into a signature of 8 polymorphisms that provide optimal prediction of T1D risk[49]. However, it is likely that the *SH2B3* sequence variant rs3184504 is not biologically responsible by itself, since sequencing studies failed to find similar SH2B3 variants in NOD mice that model many T1D features[50].

EVIDENCE FROM MOUSE MUTANTS IMPLICATES *ATXN2* IN METABOLIC SYNDROME

While the autoimmune component of T1D might be explained by the SH2B3 effect on lymphocyte proliferation, some metabolic features of T1D might be exacerbated by the ataxin-2 effect on glucose and lipid metabolism. Mice with targeted deletion of Atxn2 exon 1 and frameshift in homozygous state displayed marked obesity and infertility in two independently generated mutant lines[51,52]. Hepatic lipid and glycogen accumulation was evident already at age 6 mo. As in other insulin resistance syndromes, pancreatic and blood serum insulin levels were increased, in parallel to a reduction of insulin receptor (IR) protein levels in the liver, in spite of increased IR mRNA levels. Serum cholesterol was significantly increased[52]. Although ataxin-2 is mostly localized at the rough endoplasmic reticulum and has strong effects on mRNA processing[53-59], its effect on the IR is possibly explained through interactions with the endocytic internalization machinery of receptor tyrosine kinases[60,61]. TDP-43 is an interactor protein of ataxin-2 *via* joint RNA-binding[57], was also demonstrated to regulate glucose homeostasis and fat deposition, with its levels showing direct correlation with the expression levels of the obesity gene *Tbc1d1*, while its deletion affects the splicing of apolipoprotein A-Ⅱ[62-64].

EVIDENCE FROM HUMAN MUTATIONS IMPLICATES *ATXN2* IN OBESITY

The investigation of obesity in 92 children by systematic sequencing of the *ATXN2* coding regions demonstrated a greatly increased frequency of the SNP rs695872 allele C and an overrepresentation of (CAG)-repeat sizes > 22[65]. Indeed, obesity and polyphagia were marked features of infants in middle stages of the neurodegenerative process caused by (CAG)-repeat expansions in ATXN2[66]. Thus, monogenic evidence links obesity to ATXN2 both in mice and in human. This is possibly reflected by a genome-wide SNP genotyping analysis, where SH2B3 variants were associated with low-density lipoprotein (LDL) cholesterol[67]. Interestingly, an association with obesity was also observed for the ataxin-2

binding protein 1 (A2BP1 or RBFOX1) both in a GWAS among Pima Indians and in a candidate approach among French Caucasian adults[68].

ATXN2 IS IMPORTANT FOR NEURODEGENERATIVE DISEASES

The polyglutamine (polyQ) domain at the N-terminal end of ataxin-2 normally has a size of Q22-23, usually encoded by a $(CAG)_8CAA(CAG)_4CAA(CAG)_8$ sequence in exon 1 of the *ATXN2* gene on chromosome 12q24. Its unstable expansion to large sizes beyond $(CAG)_{31}$ is the monogenic cause of an autosomal dominant multisystem atrophy of the nervous system, which was named spinocerebellar ataxia type 2[69-86]. CAG-repeat expansions with cytosine adenosine adenosine (CAA) interruptions may also manifest as Parkinson's disease[87,88]. Intermediate CAG-repeat sizes of 26-31 units, sometimes with CAA interruptions, act as polygenic risk factor for the motor-neuron disease amyotrophic lateral sclerosis[57,89]. Intermediate CAG-repeat expansions enhance also the risk for progressive supranuclear palsy[90]. Published evidence suggests that the polyglutamine expansions increase the half-life of ataxin-2 and that a gain-of-toxic-function through accumulation of ataxin-2 aggregates with sequestration of interactor proteins such as the poly(A)-binding-protein PABPC1 underlies the neurodegenerative process[57,91]. In spite of the vast evidence that excess ataxin-2 is the biological cause for neuronal death, SNP genotyping and association studies curiously found an *SH2B3* allele haplotype to be more informative and to better predict amyotrophic lateral sclerosis risk than the ATXN2 alleles[92]. This observation underscores old experiences that maximal linkage logarithm of odds scores and maximal haplotype association scores within any chromosomal region depend on random population stratification effects and on the frequency/informativity of alleles. Thus, they are not suitable for the fine mapping of disease genes.

LONGEVITY

Interestingly, the discovery set of a GWAS of exceptional longevity in centenarians detected a significant association with the *ATXN2* SNP rs653178, in parallel to several other disease associated SNPs, while the strongest effect correlated with the SNP rs2075650 at the *TOMM40*/apolipoprotein E (*APOEO* locus. TOMM40 encodes the channel forming subunit of the translocase across the mitochondrial outer membrane, while APOE encodes the apolipoprotein E, which mediates the binding and clearance of lipoprotein particles such as chylomicrons and very LDLs. Apolipoprotein E polymorphisms are the main known genetic factors associated with the risk of Alzheimer's disease[93,94]. While it remained unclear in this longevity GWAS, whether an LD effect was consistently observed also for SNPs that surround ATXN2, and whether blood cell traits, autoimmune disorders, obesity, neurodegenerative processes or vascular pathology were underlying this observation, the authors reported their observation of a reduced frequency of the *ATXN2* SNP rs653178 allele T among centenarians [with a log10(BayesFactor) of 1.2] in the light of previous ATXN2 GWAS association data with hypertension[93,94].

KIDNEY DISEASE, MICROCIRCULATION, HYPERTENSION AND CARDIOVASCULAR INFARCTION

Indeed, several independent GWAS found renal function (estimated glomerular filtration rate on the basis of cystatin c) and chronic kidney disease to be modulated by the rs653178 variant within an intron of the *ATXN2* gene in populations of European and African ancestry[5,95-97]. Also a GWAS into plasma levels of beta-2-microglobulin as a biomarker of kidney function, cardiovascular diseases and mortality reported an association with the *ATXN2* SNP rs653178[98]. Furthermore, a recent GWAS into serum urate concentrations uncovered an association with the *ATXN2* SNP rs653178[99]. The analysis of 83 candidate SNPs showed kidney disease variants to be associated with vascular phenotypes only in the case of rs653178 within the *ATXN2* gene and two SNPs at the *SH2B3* locus[100]. A GWAS studying microcirculation as measured by retinal venular caliber reported 4 loci, with only the rs10774625 SNP within an *ATXN2* intron showing also significant association with hypertension and coronary heart disease[12]. The *ATXN2* SNP rs653178 and the *SH2B3* SNP rs3184504 association with diastolic as well as systolic blood pressure, mean arterial pressure and pulse pressure was reported in three independent GWAS of populations with European and African ancestry[7,101-103]. Similarly, an association of the *SH2B3* SNP rs3184504 with diastolic and systolic blood pressure and hypertension was detected in a GWAS of 200000 individuals of European descent[104]. A GWAS association of the *ATXN2* SNP rs653178 with myocardial infarction was shown in Icelandic individuals[28]. A recent candidate SNP study replicated the association between the *SH2B3* SNP rs3184504 and coronary heart disease also in South Asian patients[105]. Thus, it appears that the 12q24 locus has a marked effect on vascular pathology.

RED BLOOD CELL TRAITS

It is unclear whether the above vascular disorders are consequences of vessel wall pathology or of blood cell pathology. It may therefore be relevant that a GWAS into the genetic basis of six traits of erythrocytes (including hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and red blood cell count) also showed associations with the 12q24 locus from *SH2B3* across the *ATXN2* gene[106].

CONCLUSION

For further mechanistic insights it will be important to

generate and characterize rodent mutants for each of the genes in the pleiotropic 12q24 disease susceptibility locus.

With the limited knowledge available so far, it is credible that SH2B3 modulates B-lymphocyte proliferation and autoimmune traits. Ataxin-2 gain-of-function is a well-established modulator of several neurodegenerative diseases, while its deficiency appears to predispose to insulin resistance, blood cholesterol elevation, hepatic glycogen and lipid accumulation with overall obesity. Thus, downstream effects of both genes might cooperate to enhance the risk for type 1 diabetes.

Since T1D is an age-associated disease, it will be important to age Atxn2-null mice beyond 6 mo to the end of their natural lifespan around 2 years. This will allow us to assess whether their obesity leads to hypertension and vascular pathology, *e.g.*, in kidneys, whether red blood cell traits are altered, and whether their longevity is abnormal. In particular, the insulin resistance/obesity/dyslipidemia/hepatosteatosis induced by *Atxn2*-null mutations should be studied regarding their long-term consequences. Mechanistically, it will be intriguing to elucidate how the RNA processing effects of ataxin-2 lead to this pathology.

In view of the polyQ expansion effects extending the protein half-life and causing a gain-of-function of ataxin-2, it is conceivable that the polyQ shrinkage sizes (Q13-21) could mediate a decreased half-life of the protein and a partial loss-of-function. Thus, these rare variants might be associated with phenotypes that were observed in the Atxn2-null mouse, such as obesity, insulin-resistance and diabetes mellitus.

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REFERENCES

1 **Polychronakos C**, Li Q. Understanding type 1 diabetes through genetics: advances and prospects. *Nat Rev Genet* 2011; **12**: 781-792 [PMID: 22005987 DOI: 10.1038/nrg3069]

2 **Noble JA**, Erlich HA. Genetics of type 1 diabetes. *Cold Spring Harb Perspect Med* 2012; **2**: a007732 [PMID: 22315720 DOI: 10.1101/cshperspect.a007732]

3 **Scherer SE**, Muzny DM, Buhay CJ, Chen R, Cree A, Ding Y, Dugan-Rocha S, Gill R, Gunaratne P, Harris RA, Hawes AC, Hernandez J, Hodgson AV, Hume J, Jackson A, Khan ZM, Kovar-Smith C, Lewis LR, Lozado RJ, Metzker ML, Milosavljevic A, Miner GR, Montgomery KT, Morgan MB, Nazareth LV, Scott G, Sodergren E, Song XZ, Steffen D, Lovering RC, Wheeler DA, Worley KC, Yuan Y, Zhang Z, Adams CQ, Ansari-Lari MA, Ayele M, Brown MJ, Chen G, Chen Z, Clerc-Blankenburg KP, Davis C, Delgado O, Dinh HH, Draper H, Gonzalez-Garay ML, Havlak P, Jackson LR, Jacob LS, Kelly SH, Li L, Li Z, Liu J, Liu W, Lu J, Maheshwari M, Nguyen BV, Okwuonu GO, Pasternak S, Perez LM, Plopper FJ, Santibanez J, Shen H, Tabor PE, Verduzco D, Waldron L, Wang Q, Williams GA, Zhang J, Zhou J, Allen CC, Amin AG, Anyalebechi V, Bailey M, Barbaria JA, Bimage KE, Bryant NP, Burch PE, Burkett CE, Burrell KL, Calderon E, Cardenas V, Carter K, Casias K, Cavazos I, Cavazos SR, Ceasar H, Chacko J, Chan SN, Chavez D, Christopoulos C, Chu J, Cockrell R, Cox CD, Dang M, Dathorne SR, David R, Davis CM, Davy-Carroll L, Deshazo DR, Donlin JE, D'Souza L, Eaves KA, Egan A, Emery-Cohen AJ, Escotto M, Flagg N, Forbes LD, Gabisi AM, Garza M, Hamilton C, Henderson N, Hernandez O, Hines S, Hogues ME, Huang M, Idlebird DG, Johnson R, Jolivet A, Jones S, Kagan R, King LM, Leal B, Lebow H, Lee S, LeVan JM, Lewis LC, London P, Lorensuhewa LM, Loulseged H, Lovett DA, Lucier A, Lucier RL, Ma J, Madu RC, Mapua P, Martindale AD, Martinez E, Massey E, Mawhiney S, Meador MG, Mendez S, Mercado C, Mercado IC, Merritt CE, Miner ZL, Minja E, Mitchell T, Mohabbat F, Mohabbat K, Montgomery B, Moore N, Morris S, Munidasa M, Ngo RN, Nguyen NB, Nickerson E, Nwaokelemeh OO, Nwokenkwo S, Obregon M, Oguh M, Oragunye N, Oviedo RJ, Parish BJ, Parker DN, Parrish J, Parks KL, Paul HA, Payton BA, Perez A, Perrin W, Pickens A, Primus EL, Pu LL, Puazo M, Quiles MM, Quiroz JB, Rabata D, Reeves K, Ruiz SJ, Shao H, Sisson I, Sonaike T, Sorelle RP, Sutton AE, Svatek AF, Svetz LA, Tamerisa KS, Taylor TR, Teague B, Thomas N, Thorn RD, Trejos ZY, Trevino BK, Ukegbu ON, Urban JB, Vasquez LI, Vera VA, Villasana DM, Wang L, Ward-Moore S, Warren JT, Wei X, White F, Williamson AL, Wleczyk R, Wooden HS, Wooden SH, Yen J, Yoon L, Yoon V, Zorrilla SE, Nelson D, Kucherlapati R, Weinstock G, Gibbs RA; Baylor College of Medicine Human Genome Sequencing Center Sequence Production Team. The finished DNA sequence of human chromosome 12. *Nature* 2006; **440**: 346-351 [PMID: 16541075 DOI: 10.1038/nature04569]

4 **Yu F**, Sabeti PC, Hardenbol P, Fu Q, Fry B, Lu X, Ghose S, Vega R, Perez A, Pasternak S, Leal SM, Willis TD, Nelson DL, Belmont J, Gibbs RA. Positive selection of a pre-expansion CAG repeat of the human SCA2 gene. *PLoS Genet* 2005; **1**: e41 [PMID: 16205789]

5 **Köttgen A**, Pattaro C, Boger CA, Fuchsberger C, Olden M, Glazer NL, Parsa A, Gao X, Yang Q, Smith AV, O'Connell JR, Li M, Schmidt H, Tanaka T, Isaacs A, Ketkar S, Hwang SJ, Johnson AD, Dehghan A, Teumer A, Pare G, Atkinson EJ, Zeller T, Lohman K, Cornelis MC, Probst-Hensch NM, Kronenberg F, Tonjes A, Hayward C, Aspelund T, Eiriksdottir G, Launer LJ, Harris TB, Rampersaud E, Mitchell BD, Arking DE, Boerwinkle E, Struchalin M, Cavalieri M, Singleton A, Giallauria F, Metter J, de Boer IH, Haritunians T, Lumley T, Siscovick D, Psaty BM, Zillikens MC, Oostra BA, Feitosa M, Province M, de Andrade M, Turner ST, Schillert A, Ziegler A, Wild PS, Schnabel RB, Wilde S, Munzel TF, Leak TS, Illig T, Klopp N, Meisinger C, Wichmann HE, Koenig W, Zgaga L, Zemunik T, Kolcic I, Minelli C, Hu FB, Johansson A, Igl W, Zaboli G, Wild SH, Wright AF, Campbell H, Ellinghaus D, Schreiber S, Aulchenko YS, Felix JF, Rivadeneira F, Uitterlinden AG, Hofman A, Imboden M, Nitsch D, Brandstatter A, Kollerits B, Kedenko L, Magi R, Stumvoll M, Kovacs P, Boban M, Campbell S, Endlich K, Volzke H, Kroemer HK, Nauck M, Volker U, Polasek O, Vitart V, Badola S, Parker AN, Ridker PM, Kardia SL, Blankenberg S, Liu Y, Curhan GC, Franke A, Rochat T, Paulweber B, Prokopenko I, Wang W, Gudnason V, Shuldiner AR, Coresh J, Schmidt R, Ferrucci L, Shlipak MG, van Duijn CM, Borecki I, Kramer BK, Rudan I, Gyllensten U, Wilson JF, Witteman JC, Pramstaller PP, Rettig R, Hastie N, Chasman DI, Kao WH, Heid IM, Fox CS. New loci associated with kidney function and chronic kidney disease. *Nat Genet* 2010; **42**: 376-384 [PMID: 20383146 DOI: 10.1038/ng.568]

6 **McMullin MF**, Wu C, Percy MJ, Tong W. A nonsynonymous LNK polymorphism associated with idiopathic erythrocytosis. *Am J Hematol* 2011; **86**: 962-964 [PMID: 21990094 DOI: 10.1002/ajh.22154]

7 **Ganesh SK**, Tragante V, Guo W, Guo Y, Lanktree MB, Smith EN, Johnson T, Castillo BA, Barnard J, Baumert J, Chang YP, Elbers CC, Farrall M, Fischer ME, Franceschini N, Gaunt TR, Gho JM, Gieger C, Gong Y, Isaacs A, Kleber ME, Mateo Leach I, McDonough CW, Meijs MF, Mellander O, Molony

CM, Nolte IM, Padmanabhan S, Price TS, Rajagopalan R, Shaffer J, Shah S, Shen H, Soranzo N, van der Most PJ, Van Iperen EP, Van Setten JA, Vonk JM, Zhang L, Beitelshees AL, Berenson GS, Bhatt DL, Boer JM, Boerwinkle E, Burkley B, Burt A, Chakravarti A, Chen W, Cooper-Dehoff RM, Curtis SP, Dreisbach A, Duggan D, Ehret GB, Fabsitz RR, Fornage M, Fox E, Furlong CE, Gansevoort RT, Hofker MH, Hovingh GK, Kirkland SA, Kottke-Marchant K, Kutlar A, Lacroix AZ, Langaee TY, Li YR, Lin H, Liu K, Maiwald S, Malik R, Murugesan G, Newton-Cheh C, O'Connell JR, Onland-Moret NC, Ouwehand WH, Palmas W, Penninx BW, Pepine CJ, Pettinger M, Polak JF, Ramachandran VS, Ranchalis J, Redline S, Ridker PM, Rose LM, Scharnag H, Schork NJ, Shimbo D, Shuldiner AR, Srinivasan SR, Stolk RP, Taylor HA, Thorand B, Trip MD, van Duijn CM, Verschuren WM, Wijmenga C, Winkelmann BR, Wyatt S, Young JH, Boehm BO, Caulfield MJ, Chasman DI, Davidson KW, Doevendans PA, Fitzgerald GA, Gums JG, Hakonarson H, Hillege HL, Illig T, Jarvik GP, Johnson JA, Kastelein JJ, Koenig W, Marz W, Mitchell BD, Murray SS, Oldehinkel AJ, Rader DJ, Reilly MP, Reiner AP, Schadt EE, Silverstein RL, Snieder H, Stanton AV, Uitterlinden AG, van der Harst P, van der Schouw YT, Samani NJ, Johnson AD, Munroe PB, de Bakker PI, Zhu X, Levy D, Keating BJ, Asselbergs FW. Loci influencing blood pressure identified using a cardiovascular gene-centric array. *Hum Mol Genet* 2013; **22**: 1663-1678 [PMID: 23303523 DOI: 10.1093/hmg/dds555]

8 **Huang J**, Johnson AD, O'Donnell CJ. PRIMe: a method for characterization and evaluation of pleiotropic regions from multiple genome-wide association studies. *Bioinformatics* 2011; **27**: 1201-1206 [PMID: 21398673 DOI: 10.1093/bioinformatics/btr116]

9 **Gateva V**, Sandling JK, Hom G, Taylor KE, Chung SA, Sun X, Ortmann W, Kosoy R, Ferreira RC, Nordmark G, Gunnarsson I, Svenungsson E, Padyukov L, Sturfelt G, Jönsen A, Bengtsson AA, Rantapää-Dahlqvist S, Baechler EC, Brown EE, Alarcón GS, Edberg JC, Ramsey-Goldman R, McGwin G, Reveille JD, Vilá LM, Kimberly RP, Manzi S, Petri MA, Lee A, Gregersen PK, Seldin MF, Rönnblom L, Criswell LA, Syvänen AC, Behrens TW, Graham RR. A large-scale replication study identifies TNIP1, PRDM1, JAZF1, UHRF1BP1 and IL10 as risk loci for systemic lupus erythematosus. *Nat Genet* 2009; **41**: 1228-1233 [PMID: 19838195 DOI: 10.1038/ng.468]

10 **Wellcome Trust Case Control Consortium**. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 2007; **447**: 661-678 [PMID: 17554300]

11 **Cooper JD**, Walker NM, Smyth DJ, Downes K, Healy BC, Todd JA. Follow-up of 1715 SNPs from the Wellcome Trust Case Control Consortium genome-wide association study in type I diabetes families. *Genes Immun* 2009; **10** Suppl 1: S85-S94 [PMID: 19956107 DOI: 10.1038/gene.2009.97]

12 **Ikram MK**, Sim X, Jensen RA, Cotch MF, Hewitt AW, Ikram MA, Wang JJ, Klein R, Klein BE, Breteler MM, Cheung N, Liew G, Mitchell P, Uitterlinden AG, Rivadeneira F, Hofman A, de Jong PT, van Duijn CM, Kao L, Cheng CY, Smith AV, Glazer NL, Lumley T, McKnight B, Psaty BM, Jonasson F, Eiriksdottir G, Aspelund T, Harris TB, Launer LJ, Taylor KD, Li X, Iyengar SK, Xi Q, Sivakumaran TA, Mackey DA, Macgregor S, Martin NG, Young TL, Bis JC, Wiggins KL, Heckbert SR, Hammond CJ, Andrew T, Fahy S, Attia J, Holliday EG, Scott RJ, Islam FM, Rotter JI, McAuley AK, Boerwinkle E, Tai ES, Gudnason V, Siscovick DS, Vingerling JR, Wong TY. Four novel Loci (19q13, 6q24, 12q24, and 5q14) influence the microcirculation in vivo. *PLoS Genet* 2010; **6**: e1001184 [PMID: 21060863 DOI: 10.1371/journal.pgen.1001184]

13 **Velazquez L**, Cheng AM, Fleming HE, Furlonger C, Vesely S, Bernstein A, Paige CJ, Pawson T. Cytokine signaling and hematopoietic homeostasis are disrupted in Lnk-deficient mice. *J Exp Med* 2002; **195**: 1599-1611 [PMID: 12070287]

14 **Takaki S**, Sauer K, Iritani BM, Chien S, Ebihara Y, Tsuji K, Takatsu K, Perlmutter RM. Control of B cell production by the adaptor protein lnk. Definition Of a conserved family of signal-modulating proteins. *Immunity* 2000; **13**: 599-609 [PMID: 11114373]

15 **Bersenev A**, Wu C, Balcerek J, Jing J, Kundu M, Blobel GA, Chikwava KR, Tong W. Lnk constrains myeloproliferative diseases in mice. *J Clin Invest* 2010; **120**: 2058-2069 [PMID: 20458146 DOI: 10.1172/JCI42032]

16 **Roberts KG**, Morin RD, Zhang J, Hirst M, Zhao Y, Su X, Chen SC, Payne-Turner D, Churchman ML, Harvey RC, Chen X, Kasap C, Yan C, Becksfort J, Finney RP, Teachey DT, Maude SL, Tse K, Moore R, Jones S, Mungall K, Birol I, Edmonson MN, Hu Y, Buetow KE, Chen IM, Carroll WL, Wei L, Ma J, Kleppe M, Levine RL, Garcia-Manero G, Larsen E, Shah NP, Devidas M, Reaman G, Smith M, Paugh SW, Evans WE, Grupp SA, Jeha S, Pui CH, Gerhard DS, Downing JR, Willman CL, Loh M, Hunger SP, Marra MA, Mullighan CG. Genetic alterations activating kinase and cytokine receptor signaling in high-risk acute lymphoblastic leukemia. *Cancer Cell* 2012; **22**: 153-166 [PMID: 22897847 DOI: 10.1016/j.ccr.2012.06.005]

17 **Perez-Garcia A**, Ambesi-Impiombato A, Hadler M, Rigo I, LeDuc CA, Kelly K, Jalas C, Paietta E, Racevskis J, Rowe JM, Tallman MS, Paganin M, Basso G, Tong W, Chung WK, Ferrando AA. Genetic loss of SH2B3 in acute lymphoblastic leukemia. *Blood* 2013; **122**: 2425-2432 [PMID: 23908464 DOI: 10.1182/blood-2013-05-500850]

18 **Oh ST**, Simonds EF, Jones C, Hale MB, Goltsev Y, Gibbs KD, Merker JD, Zehnder JL, Nolan GP, Gotlib J. Novel mutations in the inhibitory adaptor protein LNK drive JAK-STAT signaling in patients with myeloproliferative neoplasms. *Blood* 2010; **116**: 988-992 [PMID: 20404132 DOI: 10.1182/blood-2010-02-270108]

19 **Gueller S**, Hehn S, Nowak V, Gery S, Serve H, Brandts CH, Koeffler HP. Adaptor protein Lnk binds to PDGF receptor and inhibits PDGF-dependent signaling. *Exp Hematol* 2011; **39**: 591-600 [PMID: 21310211 DOI: 10.1016/j.exphem.2011.02.001]

20 **Lasho TL**, Pardanani A, Tefferi A. LNK mutations in JAK2 mutation-negative erythrocytosis. *N Engl J Med* 2010; **363**: 1189-1190 [PMID: 20843259 DOI: 10.1056/NEJMc1006966]

21 **Ha JS**, Jeon DS. Possible new LNK mutations in myeloproliferative neoplasms. *Am J Hematol* 2011; **86**: 866-868 [PMID: 21922527 DOI: 10.1002/ajh.22107]

22 **Koren-Michowitz M**, Gery S, Tabayashi T, Lin D, Alvarez R, Nagler A, Koeffler HP. SH2B3 (LNK) mutations from myeloproliferative neoplasms patients have mild loss of function against wild type JAK2 and JAK2 V617F. *Br J Haematol* 2013; **161**: 811-820 [PMID: 23590807 DOI: 10.1111/bjh.12327]

23 **Lavrikova EY**, Nikitin AG, Kuraeva TL, Peterkova VA, Tsitlidze NM, Chistiakov DA, Nosikov VV. The carriage of the type 1 diabetes-associated R262W variant of human LNK correlates with increased proliferation of peripheral blood monocytes in diabetic patients. *Pediatr Diabetes* 2011; **12**: 127-132 [PMID: 20546165 DOI: 10.1111/j.1399-5448.2010.00656.x]

24 **Li M**, Li Z, Morris DL, Rui L. Identification of SH2B2beta as an inhibitor for SH2B1- and SH2B2alpha-promoted Janus kinase-2 activation and insulin signaling. *Endocrinology* 2007; **148**: 1615-1621 [PMID: 17204555]

25 **Buza-Vidas N**, Antonchuk J, Qian H, Månsson R, Luc S, Zandi S, Anderson K, Takaki S, Nygren JM, Jensen CT, Jacobsen SE. Cytokines regulate postnatal hematopoietic stem cell expansion: opposing roles of thrombopoietin and LNK. *Genes Dev* 2006; **20**: 2018-2023 [PMID: 16882979]

26 **Suzuki N**, Yamazaki S, Ema H, Yamaguchi T, Nakauchi H, Takaki S. Homeostasis of hematopoietic stem cells regulated by the myeloproliferative disease associated-gene product Lnk/Sh2b3 via Bcl-xL. *Exp Hematol* 2012; **40**: 166-174.e3 [PMID: 22101255 DOI: 10.1016/j.exphem.2011.11.003]

27 **Wang TC**, Chiu H, Chang YJ, Hsu TY, Chiu IM, Chen L. The adaptor protein SH2B3 (Lnk) negatively regulates neurite outgrowth of PC12 cells and cortical neurons. *PLoS One* 2011; **6**: e26433 [PMID: 22028877 DOI: 10.1371/journal.pone.0026433]

28 **Gudbjartsson DF**, Bjornsdottir US, Halapi E, Helgadottir A, Sulem P, Jonsdottir GM, Thorleifsson G, Helgadottir H, Steinthorsdottir V, Stefansson H, Williams C, Hui J, Beilby J, Warrington NM, James A, Palmer LJ, Koppelman GH, Heinzmann A, Krueger M, Boezen HM, Wheatley A, Altmuller J, Shin HD, Uh ST, Cheong HS, Jonsdottir B, Gislason D, Park CS, Rasmussen LM, Porsbjerg C, Hansen JW, Backer V, Werge T, Janson C, Jönsson UB, Ng MC, Chan J, So WY, Ma R, Shah SH, Granger CB, Quyyumi AA, Levey AI, Vaccarino V, Reilly MP, Rader DJ, Williams MJ, van Rij AM, Jones GT, Trabetti E, Malerba G, Pignatti PF, Boner A, Pescollderungg L, Girelli D, Olivieri O, Martinelli N, Ludviksson BR, Ludviksdottir D, Eyjolfsson GI, Arnar D, Thorgeirsson G, Deichmann K, Thompson PJ, Wjst M, Hall IP, Postma DS, Gislason T, Gulcher J, Kong A, Jonsdottir I, Thorsteinsdottir U, Stefansson K. Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction. *Nat Genet* 2009; **41**: 342-347 [PMID: 19198610 DOI: 10.1038/ng.323]

29 **Hunt KA**, Zhernakova A, Turner G, Heap GA, Franke L, Bruinenberg M, Romanos J, Dinesen LC, Ryan AW, Panesar D, Gwilliam R, Takeuchi F, McLaren WM, Holmes GK, Howdle PD, Walters JR, Sanders DS, Playford RJ, Trynka G, Mulder CJ, Mearin ML, Verbeek WH, Trimble V, Stevens FM, O'Morain C, Kennedy NP, Kelleher D, Pennington DJ, Strachan DP, McArdle WL, Mein CA, Wapenaar MC, Deloukas P, McGinnis R, McManus R, Wijmenga C, van Heel DA. Newly identified genetic risk variants for celiac disease related to the immune response. *Nat Genet* 2008; **40**: 395-402 [PMID: 18311140 DOI: 10.1038/ng.102]

30 **Romanos J**, Barisani D, Trynka G, Zhernakova A, Bardella MT, Wijmenga C. Six new coeliac disease loci replicated in an Italian population confirm association with coeliac disease. *J Med Genet* 2009; **46**: 60-63 [PMID: 18805825 DOI: 10.1136/jmg.2008.061457]

31 **Plaza-Izurieta L**, Castellanos-Rubio A, Irastorza I, Fernández-Jimenez N, Gutierrez G, Bilbao JR. Revisiting genome wide association studies (GWAS) in coeliac disease: replication study in Spanish population and expression analysis of candidate genes. *J Med Genet* 2011; **48**: 493-496 [PMID: 21490378 DOI: 10.1136/jmg.2011.089714]

32 **Zhernakova A**, Elbers CC, Ferwerda B, Romanos J, Trynka G, Dubois PC, de Kovel CG, Franke L, Oosting M, Barisani D, Bardella MT, Joosten LA, Saavalainen P, van Heel DA, Catassi C, Netea MG, Wijmenga C. Evolutionary and functional analysis of celiac risk loci reveals SH2B3 as a protective factor against bacterial infection. *Am J Hum Genet* 2010; **86**: 970-977 [PMID: 20560212 DOI: 10.1016/j.ajhg.2010.05.004]

33 **Hinks A**, Martin P, Flynn E, Eyre S, Packham J, Barton A, Worthington J, Thomson W. Investigation of type 1 diabetes and coeliac disease susceptibility loci for association with juvenile idiopathic arthritis. *Ann Rheum Dis* 2010; **69**: 2169-2172 [PMID: 20647273 DOI: 10.1136/ard.2009.126938]

34 **Coenen MJ**, Trynka G, Heskamp S, Franke B, van Diemen CC, Smolonska J, van Leeuwen M, Brouwer E, Boezen MH, Postma DS, Platteel M, Zanen P, Lammers JW, Groen HJ, Mali WP, Mulder CJ, Tack GJ, Verbeek WH, Wolters VM, Houwen RH, Mearin ML, van Heel DA, Radstake TR, van Riel PL, Wijmenga C, Barrera P, Zhernakova A. Common and different genetic background for rheumatoid arthritis and coeliac disease. *Hum Mol Genet* 2009; **18**: 4195-4203 [PMID: 19648290 DOI: 10.1093/hmg/ddp365]

35 **Zhernakova A**, Stahl EA, Trynka G, Raychaudhuri S, Festen EA, Franke L, Westra HJ, Fehrmann RS, Kurreeman FA, Thomson B, Gupta N, Romanos J, McManus R, Ryan AW, Turner G, Brouwer E, Posthumus MD, Remmers EF, Tucci F, Toes R, Grandone E, Mazzilli MC, Rybak A, Cukrowska B, Coenen MJ, Radstake TR, van Riel PL, Li Y, de Bakker PI, Gregersen PK, Worthington J, Siminovitch KA, Klareskog L, Huizinga TW, Wijmenga C, Plenge RM. Meta-analysis of genome-wide association studies in celiac disease and rheumatoid arthritis identifies fourteen non-HLA shared loci. *PLoS Genet* 2011; **7**: e1002004 [PMID: 21383967 DOI: 10.1371/journal.pgen.1002004]

36 **Ochoa E**, Iriondo M, Bielsa A, Ruiz-Irastorza G, Estonba A, Zubiaga AM. Thrombotic antiphospholipid syndrome shows strong haplotypic association with SH2B3-ATXN2 locus. *PLoS One* 2013; **8**: e67897 [PMID: 23844121 DOI: 10.1371/journal.pone.0067897]

37 **Alcina A**, Vandenbroeck K, Otaegui D, Saiz A, Gonzalez JR, Fernandez O, Cavanillas ML, Cénit MC, Arroyo R, Alloza I, García-Barcina M, Antigüedad A, Leyva L, Izquierdo G, Lucas M, Fedetz M, Pinto-Medel MJ, Olascoaga J, Blanco Y, Comabella M, Montalban X, Urcelay E, Matesanz F. The autoimmune disease-associated KIF5A, CD226 and SH2B3 gene variants confer susceptibility for multiple sclerosis. *Genes Immun* 2010; **11**: 439-445 [PMID: 20508602 DOI: 10.1038/gene.2010.30]

38 **Eriksson N**, Tung JY, Kiefer AK, Hinds DA, Francke U, Mountain JL, Do CB. Novel associations for hypothyroidism include known autoimmune risk loci. *PLoS One* 2012; **7**: e34442 [PMID: 22493691 DOI: 10.1371/journal.pone.0034442]

39 **Jin Y**, Birlea SA, Fain PR, Ferrara TM, Ben S, Riccardi SL, Cole JB, Gowan K, Holland PJ, Bennett DC, Luiten RM, Wolkerstorfer A, van der Veen JP, Hartmann A, Eichner S, Schuler G, van Geel N, Lambert J, Kemp EH, Gawkrodger DJ, Weetman AP, Taïeb A, Jouary T, Ezzedine K, Wallace MR, McCormack WT, Picardo M, Leone G, Overbeck A, Silverberg NB, Spritz RA. Genome-wide association analyses identify 13 new susceptibility loci for generalized vitiligo. *Nat Genet* 2012; **44**: 676-680 [PMID: 22561518 DOI: 10.1038/ng.2272]

40 **Todd JA**, Walker NM, Cooper JD, Smyth DJ, Downes K, Plagnol V, Bailey R, Nejentsev S, Field SF, Payne F, Lowe CE, Szeszko JS, Hafler JP, Zeitels L, Yang JH, Vella A, Nutland S, Stevens HE, Schuilenburg H, Coleman G, Maisuria M, Meadows W, Smink LJ, Healy B, Burren OS, Lam AA, Ovington NR, Allen J, Adlem E, Leung HT, Wallace C, Howson JM, Guja C, Ionescu-Tîrgovişte C, Simmonds MJ, Heward JM, Gough SC, Dunger DB, Wicker LS, Clayton DG. Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. *Nat Genet* 2007; **39**: 857-864 [PMID: 17554260]

41 **Smyth DJ**, Plagnol V, Walker NM, Cooper JD, Downes K, Yang JH, Howson JM, Stevens H, McManus R, Wijmenga C, Heap GA, Dubois PC, Clayton DG, Hunt KA, van Heel DA, Todd JA. Shared and distinct genetic variants in type 1 diabetes and celiac disease. *N Engl J Med* 2008; **359**: 2767-2777 [PMID: 19073967 DOI: 10.1056/NEJMoa0807917]

42 **Barrett JC**, Clayton DG, Concannon P, Akolkar B, Cooper JD, Erlich HA, Julier C, Morahan G, Nerup J, Nierras C, Plagnol V, Pociot F, Schuilenburg H, Smyth DJ, Stevens H, Todd JA, Walker NM, Rich SS. Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. *Nat Genet* 2009; **41**: 703-707 [PMID: 19430480 DOI: 10.1038/ng.381]

43 **Cooper JD**, Walker NM, Healy BC, Smyth DJ, Downes K, Todd JA. Analysis of 55 autoimmune disease and type II diabetes loci: further confirmation of chromosomes 4q27, 12q13.2 and 12q24.13 as type I diabetes loci, and support for a new locus, 12q13.3-q14.1. *Genes Immun* 2009; **10** Suppl 1: S95-120 [PMID: 19956108 DOI: 10.1038/gene.2009.98]

44 **Howson JM**, Rosinger S, Smyth DJ, Boehm BO, Todd JA. Genetic analysis of adult-onset autoimmune diabetes. *Diabetes* 2011; **60**: 2645-2653 [PMID: 21873553 DOI: 10.2337/

db11-0364]
45 **Plagnol V**, Howson JM, Smyth DJ, Walker N, Hafler JP, Wallace C, Stevens H, Jackson L, Simmonds MJ, Bingley PJ, Gough SC, Todd JA. Genome-wide association analysis of autoantibody positivity in type 1 diabetes cases. *PLoS Genet* 2011; **7**: e1002216 [PMID: 21829393 DOI: 10.1371/journal.pgen.1002216]
46 **Paré G**, Ridker PM, Rose L, Barbalic M, Dupuis J, Dehghan A, Bis JC, Benjamin EJ, Shiffman D, Parker AN, Chasman DI. Genome-wide association analysis of soluble ICAM-1 concentration reveals novel associations at the NFKBIK, PNPLA3, RELA, and SH2B3 loci. *PLoS Genet* 2011; **7**: e1001374 [PMID: 21533024 DOI: 10.1371/journal.pgen.1001374]
47 **Nikitin AG**, Lavrikova EIu, Seregin IuA, Zil'berman LI, Tsitlidze NM, Kuraeva TL, Peterkova VA, Dedov II, Nosikov VV. [Association of the polymorphisms of the ERBB3 and SH2B3 genes with type 1 diabetes]. *Mol Biol* (Mosk) 2010; **44**: 257-262 [PMID: 20586186]
48 **Reddy MV**, Wang H, Liu S, Bode B, Reed JC, Steed RD, Anderson SW, Steed L, Hopkins D, She JX. Association between type 1 diabetes and GWAS SNPs in the southeast US Caucasian population. *Genes Immun* 2011; **12**: 208-212 [PMID: 21270831 DOI: 10.1038/gene.2010.70]
49 **Winkler C**, Krumsiek J, Lempainen J, Achenbach P, Grallert H, Giannopoulou E, Bunk M, Theis FJ, Bonifacio E, Ziegler AG. A strategy for combining minor genetic susceptibility genes to improve prediction of disease in type 1 diabetes. *Genes Immun* 2012; **13**: 549-555 [PMID: 22932816 DOI: 10.1038/gene.2012.36]
50 **Li YJ**, Li XY, Guo XR, Li Y, Shen BF, Shi YC, Zhang JY. Absence of SH2B3 mutation in nonobese diabetic mice. *Genet Mol Res* 2012; **11**: 1266-1271 [PMID: 22614355 DOI: 10.4238/2012.May.9.6]
51 **Kiehl TR**, Nechiporuk A, Figueroa KP, Keating MT, Huynh DP, Pulst SM. Generation and characterization of Sca2 (ataxin-2) knockout mice. *Biochem Biophys Res Commun* 2006; **339**: 17-24 [PMID: 16293225]
52 **Lastres-Becker I**, Brodesser S, Lütjohann D, Azizov M, Buchmann J, Hintermann E, Sandhoff K, Schürmann A, Nowock J, Auburger G. Insulin receptor and lipid metabolism pathology in ataxin-2 knock-out mice. *Hum Mol Genet* 2008; **17**: 1465-1481 [PMID: 18250099 DOI: 10.1093/hmg/ddn035]
53 **Satterfield TF**, Pallanck LJ. Ataxin-2 and its Drosophila homolog, ATX2, physically assemble with polyribosomes. *Hum Mol Genet* 2006; **15**: 2523-2532 [PMID: 16835262]
54 **Nonhoff U**, Ralser M, Welzel F, Piccini I, Balzereit D, Yaspo ML, Lehrach H, Krobitsch S. Ataxin-2 interacts with the DEAD/H-box RNA helicase DDX6 and interferes with P-bodies and stress granules. *Mol Biol Cell* 2007; **18**: 1385-1396 [PMID: 17392519]
55 **Tharun S**. Roles of eukaryotic Lsm proteins in the regulation of mRNA function. *Int Rev Cell Mol Biol* 2009; **272**: 149-189 [PMID: 19121818 DOI: 10.1016/S1937-6448(08)01604-3]
56 **van de Loo S**, Eich F, Nonis D, Auburger G, Nowock J. Ataxin-2 associates with rough endoplasmic reticulum. *Exp Neurol* 2009; **215**: 110-118 [PMID: 18973756 DOI: 10.1016/j.expneurol.2008.09.020]
57 **Elden AC**, Kim HJ, Hart MP, Chen-Plotkin AS, Johnson BS, Fang X, Armakola M, Geser F, Greene R, Lu MM, Padmanabhan A, Clay-Falcone D, McCluskey L, Elman L, Juhr D, Gruber PJ, Rüb U, Auburger G, Trojanowski JQ, Lee VM, Van Deerlin VM, Bonini NM, Gitler AD. Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS. *Nature* 2010; **466**: 1069-1075 [PMID: 20740007 DOI: 10.1038/nature09320]
58 **McCann C**, Holohan EE, Das S, Dervan A, Larkin A, Lee JA, Rodrigues V, Parker R, Ramaswami M. The Ataxin-2 protein is required for microRNA function and synapse-specific long-term olfactory habituation. *Proc Natl Acad Sci USA* 2011; **108**: E655-E662 [PMID: 21795609 DOI: 10.1073/pnas.1107198108]
59 **Lim C**, Allada R. ATAXIN-2 activates PERIOD translation to sustain circadian rhythms in Drosophila. *Science* 2013; **340**: 875-879 [PMID: 23687047 DOI: 10.1126/science.1234785]
60 **Nonis D**, Schmidt MH, van de Loo S, Eich F, Dikic I, Nowock J, Auburger G. Ataxin-2 associates with the endocytosis complex and affects EGF receptor trafficking. *Cell Signal* 2008; **20**: 1725-1739 [PMID: 18602463 DOI: 10.1016/j.cellsig.2008.05.018]
61 **Drost J**, Nonis D, Eich F, Leske O, Damrath E, Brunt ER, Lastres-Becker I, Heumann R, Nowock J, Auburger G. Ataxin-2 modulates the levels of Grb2 and SRC but not ras signaling. *J Mol Neurosci* 2013; **51**: 68-81 [PMID: 23335000 DOI: 10.1007/s12031-012-9949-4]
62 **Chiang PM**, Ling J, Jeong YH, Price DL, Aja SM, Wong PC. Deletion of TDP-43 down-regulates Tbc1d1, a gene linked to obesity, and alters body fat metabolism. *Proc Natl Acad Sci USA* 2010; **107**: 16320-16324 [PMID: 20660762 DOI: 10.1073/pnas.1002176107]
63 **Stallings NR**, Puttaparthi K, Dowling KJ, Luther CM, Burns DK, Davis K, Elliott JL. TDP-43, an ALS linked protein, regulates fat deposition and glucose homeostasis. *PLoS One* 2013; **8**: e71793 [PMID: 23967244 DOI: 10.1371/journal.pone.0071793]
64 **Mercado PA**, Ayala YM, Romano M, Buratti E, Baralle FE. Depletion of TDP 43 overrides the need for exonic and intronic splicing enhancers in the human apoA-II gene. *Nucleic Acids Res* 2005; **33**: 6000-6010 [PMID: 16254078 DOI: 10.1093/nar/gki897]
65 **Figueroa KP**, Farooqi S, Harrup K, Frank J, O'Rahilly S, Pulst SM. Genetic variance in the spinocerebellar ataxia type 2 (ATXN2) gene in children with severe early onset obesity. *PLoS One* 2009; **4**: e8280 [PMID: 20016785 DOI: 10.1371/journal.pone.0008280]
66 **Abdel-Aleem A**, Zaki MS. Spinocerebellar ataxia type 2 (SCA2) in an Egyptian family presenting with polyphagia and marked CAG expansion in infancy. *J Neurol* 2008; **255**: 413-419 [PMID: 18297329 DOI: 10.1007/s00415-008-0690-4]
67 **Talmud PJ**, Drenos F, Shah S, Shah T, Palmen J, Verzilli C, Gaunt TR, Pallas J, Lovering R, Li K, Casas JP, Sofat R, Kumari M, Rodriguez S, Johnson T, Newhouse SJ, Dominiczak A, Samani NJ, Caulfield M, Sever P, Stanton A, Shields DC, Padmanabhan S, Melander O, Hastie C, Delles C, Ebrahim S, Marmot MG, Smith GD, Lawlor DA, Munroe PB, Day IN, Kivimaki M, Whittaker J, Humphries SE, Hingorani AD. Gene-centric association signals for lipids and apolipoproteins identified via the HumanCVD BeadChip. *Am J Hum Genet* 2009; **85**: 628-642 [PMID: 19913121 DOI: 10.1016/j.ajhg.2009.10.014]
68 **Ma L**, Hanson RL, Traurig MT, Muller YL, Kaur BP, Perez JM, Meyre D, Fu M, Körner A, Franks PW, Kiess W, Kobes S, Knowler WC, Kovacs P, Froguel P, Shuldiner AR, Bogardus C, Baier LJ. Evaluation of A2BP1 as an obesity gene. *Diabetes* 2010; **59**: 2837-2845 [PMID: 20724578 DOI: 10.2337/db09-1604]
69 **Imbert G**, Saudou F, Yvert G, Devys D, Trottier Y, Garnier JM, Weber C, Mandel JL, Cancel G, Abbas N, Dürr A, Didierjean O, Stevanin G, Agid Y, Brice A. Cloning of the gene for spinocerebellar ataxia 2 reveals a locus with high sensitivity to expanded CAG/glutamine repeats. *Nat Genet* 1996; **14**: 285-291 [PMID: 8896557 DOI: 10.1038/ng1196-285]
70 **Pulst SM**, Nechiporuk A, Nechiporuk T, Gispert S, Chen XN, Lopes-Cendes I, Pearlman S, Starkman S, Orozco-Diaz G, Lunkes A, DeJong P, Rouleau GA, Auburger G, Korenberg JR, Figueroa C, Sahba S. Moderate expansion of a normally biallelic trinucleotide repeat in spinocerebellar ataxia type 2. *Nat Genet* 1996; **14**: 269-276 [PMID: 8896555 DOI: 10.1038/ng1196-269]
71 **Sanpei K**, Takano H, Igarashi S, Sato T, Oyake M, Sasaki H, Wakisaka A, Tashiro K, Ishida Y, Ikeuchi T, Koide R, Saito

M, Sato A, Tanaka T, Hanyu S, Takiyama Y, Nishizawa M, Shimizu N, Nomura Y, Segawa M, Iwabuchi K, Eguchi I, Tanaka H, Takahashi H, Tsuji S. Identification of the spinocerebellar ataxia type 2 gene using a direct identification of repeat expansion and cloning technique, DIRECT. *Nat Genet* 1996; **14**: 277-284 [PMID: 8896556 DOI: 10.1038/ng1196-277]

72 **Auburger G**, Diaz GO, Capote RF, Sanchez SG, Perez MP, del Cueto ME, Meneses MG, Farrall M, Williamson R, Chamberlain S. Autosomal dominant ataxia: genetic evidence for locus heterogeneity from a Cuban founder-effect population. *Am J Hum Genet* 1990; **46**: 1163-1177 [PMID: 1971152]

73 **Orozco Diaz G**, Nodarse Fleites A, Cordovés Sagaz R, Auburger G. Autosomal dominant cerebellar ataxia: clinical analysis of 263 patients from a homogeneous population in Holguín, Cuba. *Neurology* 1990; **40**: 1369-1375 [PMID: 2392220 DOI: 10.1212/WNL.40.9.1369]

74 **Gispert S**, Twells R, Orozco G, Brice A, Weber J, Heredero L, Scheufler K, Riley B, Allotey R, Nothers C. Chromosomal assignment of the second locus for autosomal dominant cerebellar ataxia (SCA2) to chromosome 12q23-24.1. *Nat Genet* 1993; **4**: 295-299 [PMID: 8358438 DOI: 10.1038/ng0793-295]

75 **Estrada R**, Galarraga J, Orozco G, Nodarse A, Auburger G. Spinocerebellar ataxia 2 (SCA2): morphometric analyses in 11 autopsies. *Acta Neuropathol* 1999; **97**: 306-310 [PMID: 10090679 DOI: 10.1007/s004010050989]

76 **Lastres-Becker I**, Rüb U, Auburger G. Spinocerebellar ataxia 2 (SCA2). *Cerebellum* 2008; **7**: 115-124 [PMID: 18418684 DOI: 10.1007/s12311-008-0019-y]

77 **Rüb U**, Schöls L, Paulson H, Auburger G, Kermer P, Jen JC, Seidel K, Korf HW, Deller T. Clinical features, neurogenetics and neuropathology of the polyglutamine spinocerebellar ataxias type 1, 2, 3, 6 and 7. *Prog Neurobiol* 2013; **104**: 38-66 [PMID: 23438480 DOI: 10.1016/j.pneurobio.2013.01.001]

78 **Gierga K**, Bürk K, Bauer M, Orozco Diaz G, Auburger G, Schultz C, Vuksic M, Schöls L, de Vos RA, Braak H, Deller T, Rüb U. Involvement of the cranial nerves and their nuclei in spinocerebellar ataxia type 2 (SCA2). *Acta Neuropathol* 2005; **109**: 617-631 [PMID: 15906049 DOI: 10.1007/s00401-005-1014-8]

79 **Velázquez-Pérez L**, Seifried C, Santos-Falcón N, Abele M, Ziemann U, Almaguer LE, Martínez-Góngora E, Sánchez-Cruz G, Canales N, Pérez-González R, Velázquez-Manresa M, Viebahn B, von Stuckrad-Barre S, Fetter M, Klockgether T, Auburger G. Saccade velocity is controlled by polyglutamine size in spinocerebellar ataxia 2. *Ann Neurol* 2004; **56**: 444-447 [PMID: 15349876 DOI: 10.1002/ana.20220]

80 **Rüb U**, Del Turco D, Del Tredici K, de Vos RA, Brunt ER, Reifenberger G, Seifried C, Schultz C, Auburger G, Braak H. Thalamic involvement in a spinocerebellar ataxia type 2 (SCA2) and a spinocerebellar ataxia type 3 (SCA3) patient, and its clinical relevance. *Brain* 2003; **126**: 2257-2272 [PMID: 12847080 DOI: 10.1093/brain/awg234]

81 **Hernández A**, Magariño C, Gispert S, Santos N, Lunkes A, Orozco G, Heredero L, Beckmann J, Auburger G. Genetic mapping of the spinocerebellar ataxia 2 (SCA2) locus on chromosome 12q23-q24.1. *Genomics* 1995; **25**: 433-435 [PMID: 7789976 DOI: 10.1016/0888-7543(95)80043-L]

82 **Gispert S**, Lunkes A, Santos N, Orozco G, Ha-Hao D, Ratzlaff T, Aguiar J, Torrens I, Heredero L, Brice A. Localization of the candidate gene D-amino acid oxidase outside the refined I-cM region of spinocerebellar ataxia 2. *Am J Hum Genet* 1995; **57**: 972-975 [PMID: 7573064]

83 **Tuin I**, Voss U, Kang JS, Kessler K, Rüb U, Nolte D, Lochmüller H, Tinschert S, Claus D, Krakow K, Pflug B, Steinmetz H, Auburger G. Stages of sleep pathology in spinocerebellar ataxia type 2 (SCA2). *Neurology* 2006; **67**: 1966-1972 [PMID: 17159102 DOI: 10.1212/01.wnl.0000247054.90322.14]

84 **Riess O**, Laccone FA, Gispert S, Schöls L, Zühlke C, Vieira-Saecker AM, Herlt S, Wessel K, Epplen JT, Weber BH, Kreuz F, Chahrokh-Zadeh S, Meindl A, Lunkes A, Aguiar J, Macek M, Krebsová A, Macek M, Bürk K, Tinschert S, Schreyer I, Pulst SM, Auburger G. SCA2 trinucleotide expansion in German SCA patients. *Neurogenetics* 1997; **1**: 59-64 [PMID: 10735276 DOI: 10.1007/s100480050009]

85 **Belal S**, Cancel G, Stevanin G, Hentati F, Khati C, Ben Hamida C, Auburger G, Agid Y, Ben Hamida M, Brice A. Clinical and genetic analysis of a Tunisian family with autosomal dominant cerebellar ataxia type 1 linked to the SCA2 locus. *Neurology* 1994; **44**: 1423-1426 [PMID: 8058142 DOI: 10.1212/WNL.44.8.1423]

86 **Schöls L**, Gispert S, Vorgerd M, Menezes Vieira-Saecker AM, Blanke P, Auburger G, Amoiridis G, Meves S, Epplen JT, Przuntek H, Pulst SM, Riess O. Spinocerebellar ataxia type 2. Genotype and phenotype in German kindreds. *Arch Neurol* 1997; **54**: 1073-1080 [PMID: 9311350 DOI: 10.1001/archneur.1997.00550210011007]

87 **Charles P**, Camuzat A, Benammar N, Sellal F, Destée A, Bonnet AM, Lesage S, Le Ber I, Stevanin G, Dürr A, Brice A. Are interrupted SCA2 CAG repeat expansions responsible for parkinsonism? *Neurology* 2007; **69**: 1970-1975 [PMID: 17568014 DOI: 10.1212/01.wnl.0000269323.21969.db]

88 **Gispert S**, Kurz A, Waibel S, Bauer P, Liepelt I, Geisen C, Gitler AD, Becker T, Weber M, Berg D, Andersen PM, Krüger R, Riess O, Ludolph AC, Auburger G. The modulation of Amyotrophic Lateral Sclerosis risk by ataxin-2 intermediate polyglutamine expansions is a specific effect. *Neurobiol Dis* 2012; **45**: 356-361 [PMID: 21889984 DOI: 10.1016/j.nbd.2011.08.021]

89 **Lee T**, Li YR, Ingre C, Weber M, Grehl T, Gredal O, de Carvalho M, Meyer T, Tysnes OB, Auburger G, Gispert S, Bonini NM, Andersen PM, Gitler AD. Ataxin-2 intermediate-length polyglutamine expansions in European ALS patients. *Hum Mol Genet* 2011; **20**: 1697-1700 [PMID: 21292779 DOI: 10.1093/hmg/ddr045]

90 **Ross OA**, Rutherford NJ, Baker M, Soto-Ortolaza AI, Carrasquillo MM, DeJesus-Hernandez M, Adamson J, Li M, Volkening K, Finger E, Seeley WW, Hatanpaa KJ, Lomen-Hoerth C, Kertesz A, Bigio EH, Lippa C, Woodruff BK, Knopman DS, White CL, Van Gerpen JA, Meschia JF, Mackenzie IR, Boylan K, Boeve BF, Miller BL, Strong MJ, Uitti RJ, Younkin SG, Graff-Radford NR, Petersen RC, Wszolek ZK, Dickson DW, Rademakers R. Ataxin-2 repeat-length variation and neurodegeneration. *Hum Mol Genet* 2011; **20**: 3207-3212 [PMID: 21610160 DOI: 10.1093/hmg/ddr227]

91 **Damrath E**, Heck MV, Gispert S, Azizov M, Nowock J, Seifried C, Rüb U, Walter M, Auburger G. ATXN2-CAG42 sequesters PABPC1 into insolubility and induces FBXW8 in cerebellum of old ataxic knock-in mice. *PLoS Genet* 2012; **8**: e1002920 [PMID: 22956915 DOI: 10.1371/journal.pgen.1002920]

92 **Lahut S**, Ömür Ö, Uyan Ö, Ağım ZS, Özoğuz A, Parman Y, Deymeer F, Oflazer P, Koç F, Özçelik H, Auburger G, Başak AN. ATXN2 and its neighbouring gene SH2B3 are associated with increased ALS risk in the Turkish population. *PLoS One* 2012; **7**: e42956 [PMID: 22916186 DOI: 10.1371/journal.pone.0042956]

93 **Sebastiani P**, Solovieff N, Puca A, Hartley SW, Melista E, Andersen S, Dworkis DA, Wilk JB, Myers RH, Steinberg MH, Montano M, Baldwin CT, Perls TT. Genetic signatures of exceptional longevity in humans. *Science* 2010; **2010**: [PMID: 20595579 DOI: 10.1126/science.1190532]

94 **Sebastiani P**, Solovieff N, Dewan AT, Walsh KM, Puca A, Hartley SW, Melista E, Andersen S, Dworkis DA, Wilk JB, Myers RH, Steinberg MH, Montano M, Baldwin CT, Hoh J, Perls TT. Genetic signatures of exceptional longevity in humans. *PLoS One* 2012; **7**: e29848 [PMID: 22279548 DOI: 10.1371/journal.pone.0029848]

95 **Köttgen A**. Genome-wide association studies in nephrology research. *Am J Kidney Dis* 2010; **56**: 743-758 [PMID: 20728256 DOI: 10.1053/j.ajkd.2010.05.018]

96 **Böger CA**, Heid IM. Chronic kidney disease: novel insights from genome-wide association studies. *Kidney Blood Press Res* 2011; **34**: 225-234 [PMID: 21691125 DOI: 10.1159/000326901]

97 **Liu CT**, Garnaas MK, Tin A, Kottgen A, Franceschini N, Peralta CA, de Boer IH, Lu X, Atkinson E, Ding J, Nalls M, Shriner D, Coresh J, Kutlar A, Bibbins-Domingo K, Siscovick D, Akylbekova E, Wyatt S, Astor B, Mychaleckjy J, Li M, Reilly MP, Townsend RR, Adeyemo A, Zonderman AB, de Andrade M, Turner ST, Mosley TH, Harris TB, Rotimi CN, Liu Y, Kardia SL, Evans MK, Shlipak MG, Kramer H, Flessner MF, Dreisbach AW, Goessling W, Cupples LA, Kao WL, Fox CS. Genetic association for renal traits among participants of African ancestry reveals new loci for renal function. *PLoS Genet* 2011; **7**: e1002264 [PMID: 21931561 DOI: 10.1371/journal.pgen.1002264]

98 **Tin A**, Astor BC, Boerwinkle E, Hoogeveen RC, Coresh J, Kao WH. Genome-wide association study identified the human leukocyte antigen region as a novel locus for plasma beta-2 microglobulin. *Hum Genet* 2013; **132**: 619-627 [PMID: 23417110 DOI: 10.1007/s00439-013-1274-7]

99 **Köttgen A,** Albrecht E, Teumer A, Vitart V, Krumsiek J, Hundertmark C, Pistis G, Ruggiero D, O'Seaghdha CM, Haller T, Yang Q, Tanaka T, Johnson AD, Kutalik Z, Smith AV, Shi J, Struchalin M, Middelberg RP, Brown MJ, Gaffo AL, Pirastu N, Li G, Hayward C, Zemunik T, Huffman J, Yengo L, Zhao JH, Demirkan A, Feitosa MF, Liu X, Malerba G, Lopez LM, van der Harst P, Li X, Kleber ME, Hicks AA, Nolte IM, Johansson A, Murgia F, Wild SH, Bakker SJ, Peden JF, Dehghan A, Steri M, Tenesa A, Lagou V, Salo P, Mangino M, Rose LM, Lehtimaki T, Woodward OM, Okada Y, Tin A, Muller C, Oldmeadow C, Putku M, Czamara D, Kraft P, Frogheri L, Thun GA, Grotevendt A, Gislason GK, Harris TB, Launer LJ, McArdle P, Shuldiner AR, Boerwinkle E, Coresh J, Schmidt H, Schallert M, Martin NG, Montgomery GW, Kubo M, Nakamura Y, Tanaka T, Munroe PB, Samani NJ, Jacobs DR, Jr., Liu K, D'Adamo P, Ulivi S, Rotter JI, Psaty BM, Vollenweider P, Waeber G, Campbell S, Devuyst O, Navarro P, Kolcic I, Hastie N, Balkau B, Froguel P, Esko T, Salumets A, Khaw KT, Langenberg C, Wareham NJ, Isaacs A, Kraja A, Zhang Q, Wild PS, Scott RJ, Holliday EG, Org E, Viigimaa M, Bandinelli S, Metter JE, Lupo A, Trabetti E, Sorice R, Doring A, Lattka E, Strauch K, Theis F, Waldenberger M, Wichmann HE, Davies G, Gow AJ, Bruinenberg M, Stolk RP, Kooner JS, Zhang W, Winkelmann BR, Boehm BO, Lucae S, Penninx BW, Smit JH, Curhan G, Mudgal P, Plenge RM, Portas L, Persico I, Kirin M, Wilson JF, Mateo Leach I, van Gilst WH, Goel A, Ongen H, Hofman A, Rivadeneira F, Uitterlinden AG, Imboden M, von Eckardstein A, Cucca F, Nagaraja R, Piras MG, Nauck M, Schurmann C, Budde K, Ernst F, Farrington SM, Theodoratou E, Prokopenko I, Stumvoll M, Jula A, Perola M, Salomaa V, Shin SY, Spector TD, Sala C, Ridker PM, Kahonen M, Viikari J, Hengstenberg C, Nelson CP, Meschia JF, Nalls MA, Sharma P, Singleton AB, Kamatani N, Zeller T, Burnier M, Attia J, Laan M, Klopp N, Hillege HL, Kloiber S, Choi H, Pirastu M, Tore S, Probst-Hensch NM, Volzke H, Gudnason V, Parsa A, Schmidt R, Whitfield JB, Fornage M, Gasparini P, Siscovick DS, Polasek O, Campbell H, Rudan I, Bouatia-Naji N, Metspalu A, Loos RJ, van Duijn CM, Borecki IB, Ferrucci L, Gambaro G, Deary IJ, Wolffenbuttel BH, Chambers JC, Marz W, Pramstaller PP, Snieder H, Gyllensten U, Wright AF, Navis G, Watkins H, Witteman JC, Sanna S, Schipf S, Dunlop MG, Tonjes A, Ripatti S, Soranzo N, Toniolo D, Chasman DI, Raitakari O, Kao WH, Ciullo M, Fox CS, Caulfield M, Bochud M, Gieger C. Genome-wide association analyses identify 18 new loci associated with serum urate concentrations. *Nat Genet* 2013; **45**: 145-154 [PMID: 23263486 DOI: 10.1038/ng.2500]

100 **Olden M**, Teumer A, Bochud M, Pattaro C, Köttgen A, Turner ST, Rettig R, Chen MH, Dehghan A, Bastardot F, Schmidt R, Vollenweider P, Schunkert H, Reilly MP, Fornage M, Launer LJ, Verwoert GC, Mitchell GF, Bis JC, O'Donnell CJ, Cheng CY, Sim X, Siscovick DS, Coresh J, Kao WH, Fox CS, O'Seaghdha CM. Overlap between common genetic polymorphisms underpinning kidney traits and cardiovascular disease phenotypes: the CKDGen consortium. *Am J Kidney Dis* 2013; **61**: 889-898 [PMID: 23474010 DOI: 10.1053/j.ajkd.2012.12.024]

101 **Levy D**, Ehret GB, Rice K, Verwoert GC, Launer LJ, Dehghan A, Glazer NL, Morrison AC, Johnson AD, Aspelund T, Aulchenko Y, Lumley T, Köttgen A, Vasan RS, Rivadeneira F, Eiriksdottir G, Guo X, Arking DE, Mitchell GF, Mattace-Raso FU, Smith AV, Taylor K, Scharpf RB, Hwang SJ, Sijbrands EJ, Bis J, Harris TB, Ganesh SK, O'Donnell CJ, Hofman A, Rotter JI, Coresh J, Benjamin EJ, Uitterlinden AG, Heiss G, Fox CS, Witteman JC, Boerwinkle E, Wang TJ, Gudnason V, Larson MG, Chakravarti A, Psaty BM, van Duijn CM. Genome-wide association study of blood pressure and hypertension. *Nat Genet* 2009; **41**: 677-687 [PMID: 19430479 DOI: 10.1038/ng.384]

102 **Newton-Cheh C**, Johnson T, Gateva V, Tobin MD, Bochud M, Coin L, Najjar SS, Zhao JH, Heath SC, Eyheramendy S, Papadakis K, Voight BF, Scott LJ, Zhang F, Farrall M, Tanaka T, Wallace C, Chambers JC, Khaw KT, Nilsson P, van der Harst P, Polidoro S, Grobbee DE, Onland-Moret NC, Bots ML, Wain LV, Elliott KS, Teumer A, Luan J, Lucas G, Kuusisto J, Burton PR, Hadley D, McArdle WL, Brown M, Dominiczak A, Newhouse SJ, Samani NJ, Webster J, Zeggini E, Beckmann JS, Bergmann S, Lim N, Song K, Vollenweider P, Waeber G, Waterworth DM, Yuan X, Groop L, Orho-Melander M, Allione A, Di Gregorio A, Guarrera S, Panico S, Ricceri F, Romanazzi V, Sacerdote C, Vineis P, Barroso I, Sandhu MS, Luben RN, Crawford GJ, Jousilahti P, Perola M, Boehnke M, Bonnycastle LL, Collins FS, Jackson AU, Mohlke KL, Stringham HM, Valle TT, Willer CJ, Bergman RN, Morken MA, Doring A, Gieger C, Illig T, Meitinger T, Org E, Pfeufer A, Wichmann HE, Kathiresan S, Marrugat J, O'Donnell CJ, Schwartz SM, Siscovick DS, Subirana I, Freimer NB, Hartikainen AL, McCarthy MI, O'Reilly PF, Peltonen L, Pouta A, de Jong PE, Snieder H, van Gilst WH, Clarke R, Goel A, Hamsten A, Peden JF, Seedorf U, Syvanen AC, Tognoni G, Lakatta EG, Sanna S, Scheet P, Schlessinger D, Scuteri A, Dorr M, Ernst F, Felix SB, Homuth G, Lorbeer R, Reffelmann T, Rettig R, Volker U, Galan P, Gut IG, Hercberg S, Lathrop GM, Zelenika D, Deloukas P, Soranzo N, Williams FM, Zhai G, Salomaa V, Laakso M, Elosua R, Forouhi NG, Volzke H, Uiterwaal CS, van der Schouw YT, Numans ME, Matullo G, Navis G, Berglund G, Bingham SA, Kooner JS, Connell JM, Bandinelli S, Ferrucci L, Watkins H, Spector TD, Tuomilehto J, Altshuler D, Strachan DP, Laan M, Meneton P, Wareham NJ, Uda M, Jarvelin MR, Mooser V, Melander O, Loos RJ, Elliott P, Abecasis GR, Caulfield M, Munroe PB. Genome-wide association study identifies eight loci associated with blood pressure. *Nat Genet* 2009; **41**: 666-676 [PMID: 19430483 DOI: 10.1038/ng.361]

103 **Fox ER**, Young JH, Li Y, Dreisbach AW, Keating BJ, Musani SK, Liu K, Morrison AC, Ganesh S, Kutlar A, Ramachandran VS, Polak JF, Fabsitz RR, Dries DL, Farlow DN, Redline S, Adeyemo A, Hirschorn JN, Sun YV, Wyatt SB, Penman AD, Palmas W, Rotter JI, Townsend RR, Doumatey AP, Tayo BO, Mosley TH, Lyon HN, Kang SJ, Rotimi CN, Cooper RS, Franceschini N, Curb JD, Martin LW, Eaton CB, Kardia SL, Taylor HA, Caulfield MJ, Ehret GB, Johnson T, Chakravarti A, Zhu X, Levy D. Association of genetic variation with systolic and diastolic blood pressure among African Americans: the Candidate Gene Association Resource study. *Hum Mol Genet* 2011; **20**: 2273-2284 [PMID: 21378095 DOI: 10.1093/hmg/ddr092]

104 **International Consortium for Blood Pressure Genome-Wide Association Studies;** Ehret GB, Munroe PB, Rice KM, Bochud M, Johnson AD, Chasman DI, Smith AV, Tobin MD,

Verwoert GC, Hwang SJ, Pihur V, Vollenweider P, O'Reilly PF, Amin N, Bragg-Gresham JL, Teumer A, Glazer NL, Launer L, Zhao JH, Aulchenko Y, Heath S, Sober S, Parsa A, Luan J, Arora P, Dehghan A, Zhang F, Lucas G, Hicks AA, Jackson AU, Peden JF, Tanaka T, Wild SH, Rudan I, Igl W, Milaneschi Y, Parker AN, Fava C, Chambers JC, Fox ER, Kumari M, Go MJ, van der Harst P, Kao WH, Sjogren M, Vinay DG, Alexander M, Tabara Y, Shaw-Hawkins S, Whincup PH, Liu Y, Shi G, Kuusisto J, Tayo B, Seielstad M, Sim X, Nguyen KD, Lehtimaki T, Matullo G, Wu Y, Gaunt TR, Onland-Moret NC, Cooper MN, Platou CG, Org E, Hardy R, Dahgam S, Palmen J, Vitart V, Braund PS, Kuznetsova T, Uiterwaal CS, Adeyemo A, Palmas W, Campbell H, Ludwig B, Tomaszewski M, Tzoulaki I, Palmer ND, Aspelund T, Garcia M, Chang YP, O'Connell JR, Steinle NI, Grobbee DE, Arking DE, Kardia SL, Morrison AC, Hernandez D, Najjar S, McArdle WL, Hadley D, Brown MJ, Connell JM, Hingorani AD, Day IN, Lawlor DA, Beilby JP, Lawrence RW, Clarke R, Hopewell JC, Ongen H, Dreisbach AW, Li Y, Young JH, Bis JC, Kahonen M, Viikari J, Adair LS, Lee NR, Chen MH, Olden M, Pattaro C, Bolton JA, Kottgen A, Bergmann S, Mooser V, Chaturvedi N, Frayling TM, Islam M, Jafar TH, Erdmann J, Kulkarni SR, Bornstein SR, Grassler J, Groop L, Voight BF, Kettunen J, Howard P, Taylor A, Guarrera S, Ricceri F, Emilsson V, Plump A, Barroso I, Khaw KT, Weder AB, Hunt SC, Sun YV, Bergman RN, Collins FS, Bonnycastle LL, Scott LJ, Stringham HM, Peltonen L, Perola M, Vartiainen E, Brand SM, Staessen JA, Wang TJ, Burton PR, Soler Artigas M, Dong Y, Snieder H, Wang X, Zhu H, Lohman KK, Rudock ME, Heckbert SR, Smith NL, Wiggins KL, Doumatey A, Shriner D, Veldre G, Viigimaa M, Kinra S, Prabhakaran D, Tripathy V, Langefeld CD, Rosengren A, Thelle DS, Corsi AM, Singleton A, Forrester T, Hilton G, McKenzie CA, Salako T, Iwai N, Kita Y, Ogihara T, Ohkubo T, Okamura T, Ueshima H, Umemura S, Eyheramendy S, Meitinger T, Wichmann HE, Cho YS, Kim HL, Lee JY, Scott J, Sehmi JS, Zhang W, Hedblad B, Nilsson P, Smith GD, Wong A, Narisu N, Stancakova A, Raffel LJ, Yao J, Kathiresan S, O'Donnell CJ, Schwartz SM, Ikram MA, Longstreth WT, Jr., Mosley TH, Seshadri S, Shrine NR, Wain LV, Morken MA, Swift AJ, Laitinen J, Prokopenko I, Zitting P, Cooper JA, Humphries SE, Danesh J, Rasheed A, Goel A, Hamsten A, Watkins H, Bakker SJ, van Gilst WH, Janipalli CS, Mani KR, Yajnik CS, Hofman A, Mattace-Raso FU, Oostra BA, Demirkan A, Isaacs A, Rivadeneira F, Lakatta EG, Orru M, Scuteri A, Ala-Korpela M, Kangas AJ, Lyytikainen LP, Soininen P, Tukiainen T, Wurtz P, Ong RT, Dorr M, Kroemer HK, Volker U, Volzke H, Galan P, Hercberg S, Lathrop M, Zelenika D, Deloukas P, Mangino M, Spector TD, Zhai G, Meschia JF, Nalls MA, Sharma P, Terzic J, Kumar MV, Denniff M, Zukowska-Szczechowska E, Wagenknecht LE, Fowkes FG, Charchar FJ, Schwarz PE, Hayward C, Guo X, Rotimi C, Bots ML, Brand E, Samani NJ, Polasek O, Talmud PJ, Nyberg F, Kuh D, Laan M, Hveem K, Palmer LJ, van der Schouw YT, Casas JP, Mohlke KL, Vineis P, Raitakari O, Ganesh SK, Wong TY, Tai ES, Cooper RS, Laakso M, Rao DC, Harris TB, Morris RW, Dominiczak AF, Kivimaki M, Marmot MG, Miki T, Saleheen D, Chandak GR, Coresh J, Navis G, Salomaa V, Han BG, Zhu X, Kooner JS, Melander O, Ridker PM, Bandinelli S, Gyllensten UB, Wright AF, Wilson JF, Ferrucci L, Farrall M, Tuomilehto J, Pramstaller PP, Elosua R, Soranzo N, Sijbrands EJ, Altshuler D, Loos RJ, Shuldiner AR, Gieger C, Meneton P, Uitterlinden AG, Wareham NJ, Gudnason V, Rotter JI, Rettig R, Uda M, Strachan DP, Witteman JC, Hartikainen AL, Beckmann JS, Boerwinkle E, Vasan RS, Boehnke M, Larson MG, Jarvelin MR, Psaty BM, Abecasis GR, Chakravarti A, Elliott P, van Duijn CM, Newton-Cheh C, Levy D, Caulfield MJ, Johnson T. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature* 2011; **478**: 103-109 [PMID: 21909115 DOI: 10.1038/nature10405]

105 **Lian J**, Huang Y, Huang RS, Xu L, Le Y, Yang X, Xu W, Huang X, Ye M, Zhou J, Duan S. Meta-analyses of four eosinophil related gene variants in coronary heart disease. *J Thromb Thrombolysis* 2013; **36**: 394-401 [PMID: 23328882 DOI: 10.1007/s11239-012-0862-z]

106 **Ganesh SK**, Zakai NA, van Rooij FJ, Soranzo N, Smith AV, Nalls MA, Chen MH, Kottgen A, Glazer NL, Dehghan A, Kuhnel B, Aspelund T, Yang Q, Tanaka T, Jaffe A, Bis JC, Verwoert GC, Teumer A, Fox CS, Guralnik JM, Ehret GB, Rice K, Felix JF, Rendon A, Eiriksdottir G, Levy D, Patel KV, Boerwinkle E, Rotter JI, Hofman A, Sambrook JG, Hernandez DG, Zheng G, Bandinelli S, Singleton AB, Coresh J, Lumley T, Uitterlinden AG, Vangils JM, Launer LJ, Cupples LA, Oostra BA, Zwaginga JJ, Ouwehand WH, Thein SL, Meisinger C, Deloukas P, Nauck M, Spector TD, Gieger C, Gudnason V, van Duijn CM, Psaty BM, Ferrucci L, Chakravarti A, Greinacher A, O'Donnell CJ, Witteman JC, Furth S, Cushman M, Harris TB, Lin JP. Multiple loci influence erythrocyte phenotypes in the CHARGE Consortium. *Nat Genet* 2009; **41**: 1191-1198 [PMID: 19862010 DOI: 10.1038/ng.466]

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REVIEW

Interrelationships between ghrelin, insulin and glucose homeostasis: Physiological relevance

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Abstract

Ghrelin is a 28 amino acid peptide mainly derived from the oxyntic gland of the stomach. Both acylated (AG) and unacylated (UAG) forms of ghrelin are found in the circulation. Initially, AG was considered as the only bioactive form of ghrelin. However, recent advances indicate that both AG and UAG exert distinct and common effects in organisms. Soon after its discovery, ghrelin was shown to promote appetite and adiposity in animal and human models. In response to these anabolic effects, an impressive number of elements have suggested the influence of ghrelin on the regulation of metabolic functions and the development of obesity-related disorders. However, due to the complexity of its biochemical nature and the physiological processes it governs, some of the effects of ghrelin are still debated in the literature. Evidence suggests that ghrelin influences glucose homeostasis through the modulation of insulin secretion and insulin receptor signaling. On the other hand, insulin was also shown to influence circulating levels of ghrelin. Here, we review the relationship between ghrelin and insulin and we describe the impact of this interaction on the modulation of glucose homeostasis.

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Key words: Acylated ghrelin; Unacylated ghrelin; Insulin secretion; β-cell functions; Insulin receptor signalling; Glucose homeostasis

Core tip: The present invited review intends to summarize the current knowledge on the relationships between ghrelin, insulin and glucose homeostasis in cellular, animal and human models.

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INTRODUCTION

Obesity and ensuing metabolic complications are major concerns for public health and these disturbances are anticipated to cause the first reduction of life expectancy in modern history[1]. Unfortunately, efforts to curb and especially prevent this alarming trend have so far been met with disappointment. Although it was initially hypothesized that metabolic dysfunctions develop in response to overeating and sedentarity, recent advances show that the

pathophysiological process is much more complex than anticipated. That is, obesogenic environmental and genetic factors disturb homeostatic crosstalk between tissues, promote excessive fat deposition and ultimately alter cellular functions[2-7]. Recently, a close relationship between the development of obesity-related disturbances and gut-derived hormonal dysregulations has been clearly established[8-11]. For instance, studies of gut-derived peptides such as peptide tyrosine-tyrosine 3-36, glucagon-like peptide 1, glucose-dependent insulinotropic peptide and oxyntomodulin have provided key information regarding factors promoting satiety, insulin secretion and glucose disposal. More recently, studies on ghrelin have significantly improved our understanding of mechanisms underlying the stimulation of food intake, lipid accumulation in adipose tissues and the development of metabolic dysfunctions such as insulin resistance and type 2 diabetes[12].

Ghrelin is a 28 amino acid peptide predominantly produced by the stomach[13-15] but also expressed at lower levels in other tissues such as the liver, pancreas, heart, central nervous system (CNS), esophagus and testis[16-18]. Although it was isolated from rat stomach extracts[13] ghrelin was initially shown to induce potent somatotrophic activity in the anterior pituitary[19-21]. Subsequent studies have also revealed the relevance of ghrelin in the regulation of appetite, storage and metabolism of energy substrates, inflammation, stress and other key biological functions[22,23]. Strong evidence indicates the effects of ghrelin in the regulation of metabolic functions and its potential role in the etiology of obesity-related dysfunctions such as insulin resistance and type 2 diabetes[24]. For the purpose of the present work, we will emphasize on reviewing the inter-relationships between ghrelin, insulin and glucose homeostasis.

GHRELIN RECEPTOR

In the circulation, ghrelin is present under acylated (AG) and unacylated (UAG) forms[13]. The enzyme ghrelin o-acyltransferase (GOAT) was shown to be mandatory for the posttranslational addition of the acyl chain on serine-3 of ghrelin[25]. In blood, the half-life of AG is approximately 10 min while UAG displays more stability with a half-life of more than 35 min[26]. Although UAG accounts for approximately 50%-90% of total ghrelin concentrations in the circulation, this form was initially considered as an artifact devoid of biological activity[26,27]. However, recent advances indicate that UAG independently mediates specific biological functions while sharing others with AG.

The effects of AG are mediated through the activation of the native growth hormone (GH) secretagogue receptor 1a (GHS-R1a)[13,28]. Following the discovery of ghrelin, the AG form was reported to stimulate the release of GH and to promote appetite through its action on the brain[13,29-31]. In contrast to its acylated counterpart, UAG was not shown to interact with the GHS-R1a. It has recently been suggested that AG and UAG may exert their effects through the interaction with other receptors than the already identified GHS-R1a. The human ghrelin analog BIM-28163, which fully inhibits GHS-R1a receptor activation induced by native ghrelin, was shown to blunt AG-induced GH secretion[32]. However, since both AG and BIM-28163 induce neuronal activation in the dorsomedial hypothalamus, an important nucleus involved in regulating food intake, it is suggested that an unknown ghrelin receptor could mediate AG's action in promoting weight gain[33,34]. Accordingly, it is proposed that the GHS-R1a acutely mediates AG action on appetite, whereas an unknown ghrelin receptor modulates its chronic peripheral weight-increasing effects[35,36]. It has also been suggested that GHS-R1a could heterodimerize with G protein-coupled receptor 83 (Gpr83)[37]. This study shows that the Gpr83/GHS-R1a dimerization affects ghrelin's ability to activate its only known endogenous receptor, indicating that Gpr83 is an important regulator of ghrelin receptor activity. AG was also shown to interact with several other G protein-coupled receptors such as the dopamine receptor subtypes 1 and 2 (DRD1/2) and melanocortin receptor 3 (MC3R) in the central nervous system[37-41]. Because the existence of another ghrelin receptor remains speculative, the following sections will emphasize on the interactions between GHS-R1a and insulin synthesis/release and signalling.

In a landmark article, Tschöp *et al*[30] had observed that AG increases both food intake and adiposity in rats and mice, suggesting that the hormone promotes positive energy balance. GHS-R1a is predominantly expressed in the central areas known to be influenced by insulin, including hypothalamic neuropeptide Y (NPY)/agouti-related protein (AgRP) neurons[42,43]. Furthermore, we and others have reported that the orexigenic effects of AG are mediated through the activation of NPY and AgRP as well as the inhibition of proopiomelanocortin (POMC)/cocaine- and amphetamine-regulated transcript (CART) neurons in the arcuate nucleus (ARC) of the hypothalamus[29,44-49]. It has recently been hypothesized that the adipogenic effects of both AG and UAG could be mediated in the CNS by the activation of GHS-R1a[50]. Mice lacking GHS-R1a are protected against early-onset obesity, indicating the importance of ghrelin signaling in regulating body weight[51]. The effect of AG on food intake is believed to be mainly attributable to its interaction with the melanocortin system[44,52]. In fact, in the hypothalamus, ghrelin promotes the expression of the enzyme prolylcarboxypeptidase and therefore the degradation of melanocortin receptor agonist α-melanocyte-stimulating hormone[53]. Central melanocortin signaling has been shown to directly regulate insulin levels and to be independently involved in the control of glucose homeostasis[54]. Moreover, the melanocortin system is an important downstream target for the effects of insulin to regulate food intake and body weight[55]. The melanocortin system is active in areas where both insulin and ghrelin signalling components are expressed; therefore, potential crosstalks between these systems could be envisaged.

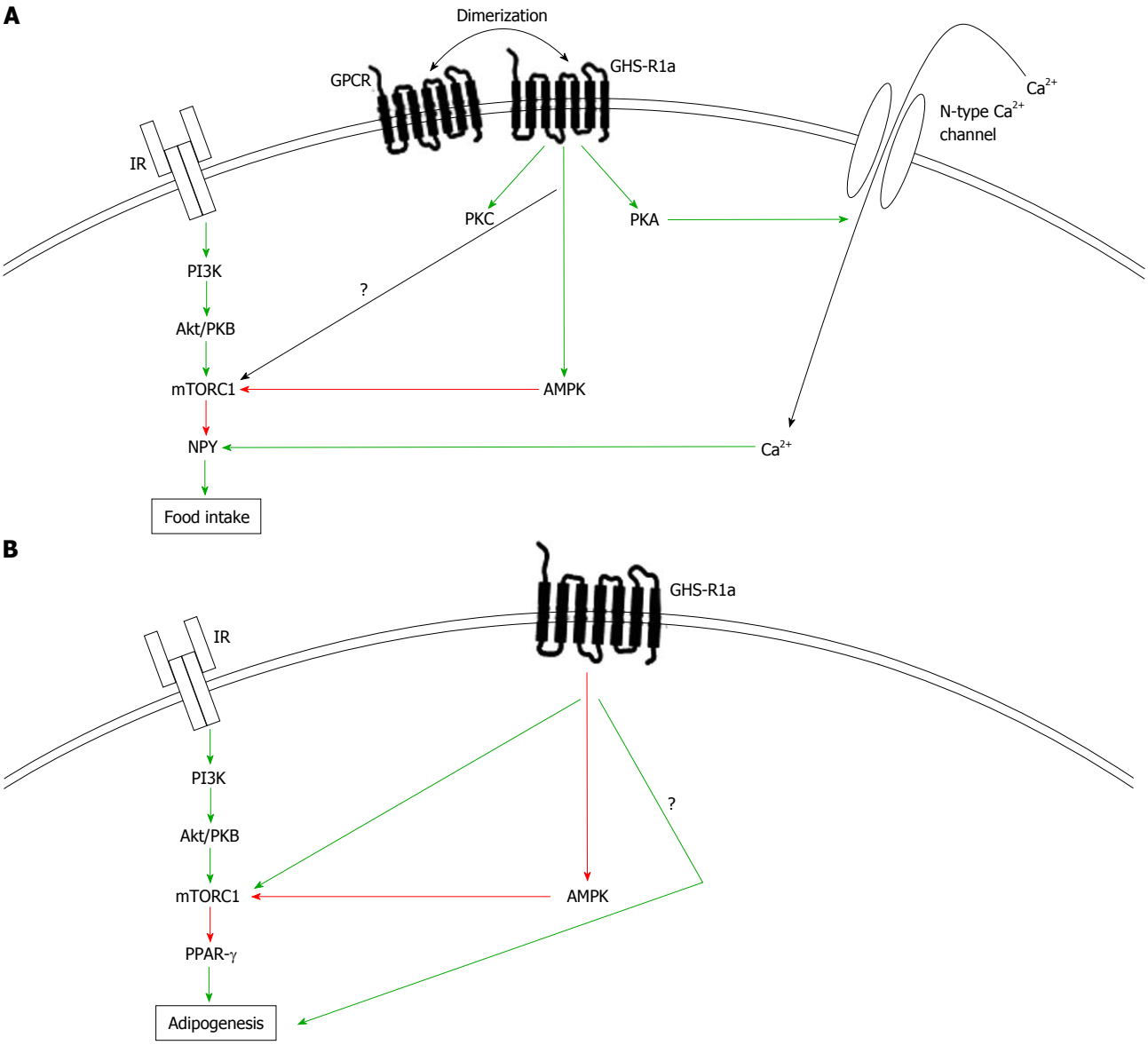

Figure 1 Crosstalks between ghrelin and insulin signaling. A: In the CNS, the interaction between GHS-R1a and ghrelin leads to the activation of PKC and PKA and ultimately to the opening of calcium channels. In the ARC, AG's orexigenic effects are solely mediated through PKA activation and the intracellular entry of Ca^{2+}; which in turn, generate a depolarization/activation of NPY neurons. GHS-R1a activation also triggers AMPK phosphorylation. Also, the activation insulin signaling pathway leads to a phosphorylation cascade that involves PI3K, Akt/PKB and mTORC1. mTORC1 has been shown to reduce food intake by inhibiting NPY expression in ARC neurons. This suggests the existence of a crosstalk between these two signaling pathways, considering that AMPK inhibits mTORC1 activation while ghrelin also reduces the anorexigenic effects of insulin-mTORC1. GHS-R1a could also mediate mTORC1 activation through an AMPK-independent mechanism. Moreover, GHS-R1a has been shown to dimerize with some GPCRs such as Gpr83, DRD1/2 and MC3R; B: In the periphery, the adipogenic effects of ghrelin have been shown to synergize with insulin signaling. In contrast to its central effects, the interaction between GHS-R1a and AG leads to decreases in AMPK activity in the periphery. GHS-R1a also activates Akt, PKB, mTORC1 and ultimately PPAR-γ to stimulate insulin-induced adipogenesis. CNS: Central nervous system; PKC: Protein kinase C; PKA: Protein kinase A; ARC: Arcuate nucleus; GHS-R1a: Growth hormone secretagogue receptor 1a; NPY: Neuropeptide Y; AG: Acylated ghrelin; AMPK: AMP-activated protein kinase; mTORC1: Mechanistic target of rapamycin complex 1; MC3R: Melanocortin receptor 3; DRD1/2: Dopamine receptor subtypes 1 and 2; Gpr83: G protein-coupled receptor 83; GPCR: G protein-coupled receptors; PPAR-γ: Peroxisome proliferator-activated receptor γ; IR: Insulin receptor.

COMMON PATHWAY FOR GHRELIN AND INSULIN RECEPTOR SIGNALING

In the central nervous system

As mentioned above, it is believed that the effects of ghrelin on feeding are mainly exerted through the ARC[29,56,57]. Since the central administration of ghrelin increases the mRNA expression of NPY and AgRP while inhibiting the transcription of POMC and CART, it has been suggested that the orexigenic actions of ghrelin are mediated through the activation of these neurons[29,44-49,58]. As presented in Figure 1A, GHS-R1a activation regulates intracellular calcium through the adenylate cyclase-protein kinase A (PKA) and phospholipase C-protein kinase C (PKC) pathways[43,59]. The PKA pathway has been shown to be related to the orexigenic effects of ghrelin since inhibitors of PKC do not influence the calcium response to ghrelin in NPY neurons of the ARC[43]. Consequently, GHS-R1a activation in the ARC elicits calcium signaling through N-type calcium channel-dependent mechanisms.

AMP-activated protein kinase (AMPK) plays an important role in the regulation of energy metabolism. This kinase is activated following an increase in the AMP/ATP ratio within the cell, a condition linked to cellular energy depletion[60]. Once activated, AMPK phosphorylates acetyl-CoA carboxylase and switches on catabolic processes to promote ATP production[60]. Current evidence indicates that ghrelin could be considered as a signal of energy deficiency since it activates AMPK in the CNS. Moreover, ghrelin-induced calcium entry is substantially suppressed by an AMPK inhibitor[61]. Consistent with these observations, GHS-R1a positively modulates hypothalamic AMPK[61,62]. In turn, the pharmacological activation of AMPK was also shown to stimulate food intake in the hypothalamus[62]. This reinforces the view that AMPK is critical in the control of feeding. However, little is known regarding the potential mechanisms through which AMPK-activation would mediate ghrelin's orexigenic effects. Recent data suggest that in response to fasting, increased ghrelin levels promote feeding through AMPK-mediated activation of hypothalamic fatty acid metabolism in the ventromedial hypothalamus (VMH)[63]. Further studies are needed to identify the mechanisms underlying ghrelin's activation of AMPK and to characterize the neuronal centers involved in the stimulation of appetite.

AMPK influences the insulin signaling pathway, suggesting that ghrelin-induced activation of AMPK could affect this pathway. In fact, the activation of AMPK inhibits the mechanistic target of rapamycin (mTOR) complex 1 (mTORC1) activity, a key protein complex activated downstream of the insulin receptor (IR). mTORC1 is a central regulator of cell metabolism, growth, proliferation and survival and acts as a nutrient/hormone sensor[64,65]. In the CNS, mTORC1 activation reduces food intake at least by reducing the hypothalamic expression of NPY and AgRP[66,67]. Recent data indicate that ghrelin requires an intact hypothalamic mTORC1 to stimulate food intake[68]. In this study, the authors suggest that orexigenic effect of ghrelin is mediated by AMPK in the VMH, but through the mTORC1 in the ARC. These results are rather counterintuitive since the effects of AMPK and mTORC1 usually antagonize each other. AMPK activation promotes food intake whereas mTORC1 does the opposite. Indeed, injection of insulin in rodents inhibits AMPK activity in the hypothalamus, promotes mTORC1 activation, and reduces food consumption[69]. Recently, is has been suggested that ghrelin plays a dual time-dependent role in modulating hypothalamus, since it only transiently affects AMPK, which might explain the conflicting results[70]. More studies are needed to better understand the signaling events mediating the effects of ghrelin on the regulation of food intake.

In the periphery

As indicated in Figure 1B, in contrast to its central effects, ghrelin decreases AMPK activity in the periphery, indicating that the hormone bilaterally controls AMPK in the brain and peripherally. Because of this divergence in AMPK activation between the brain and the periphery, it is expected that ghrelin and insulin signaling crosstalks will be different in the CNS versus the periphery. In the periphery, it was observed that ghrelin stimulates adipogenesis[10,22]. The adipogenic effects of ghrelin are mediated, at least in part, through the activation of peroxisome proliferator-activated receptor γ (PPAR-γ), a nuclear receptor whose activity is positively influenced by key components of the insulin pathway, namely Akt/PKB and mTORC1[71-73]. In fact in the periphery, AG promotes adipogenesis through PPAR-γ. Interestingly, a fully operational form of the mTORC1 complex is required for PPAR-γ activation; suggesting that AG's adipogenic effects could be mediated through mTORC1. Consistently, ghrelin promotes activation of the Akt/PKB pathway in macrophages, and this activation results in an enhanced activation of PPAR-γ[74]. Unlike in the CNS, GHS-R1a adipogenic actions seem to synergize with the insulin signaling pathway, establishing the need to further understand the discrepancies between mTOR, AMPK, insulin and ghrelin action in the brain versus peripheral tissues. It is noteworthy that both endogenous and pharmacological activation of AMPK prevent adipogenesis while downregulating the expression of key adipogenic genes including PPAR-γ in the periphery[75,76]. Overall, these elements suggest that ghrelin needs to inhibit peripheral AMPK to exert its effects on fat accumulation.

It is also suggested that the insulin signaling pathway and insulin *per se* can affect ghrelin production and signaling. It has been shown that components of the mTOR signaling pathway are expressed in the endocrine cells of gastric mucosa, where nearly all ghrelin-positive cells are positively stained for these signaling molecules[77]. Moreover, rapamycin, a mTORC1 inhibitor increases gastric ghrelin mRNA, gastric preproghrelin levels and circulating ghrelin, demonstrating that the mTORC1 signaling pathway is crucial in ghrelin expression and secretion[78]. Therefore, insulin could also directly affect ghrelin secretion. Altogether, these findings strongly suggest the existence of a link between ghrelin and insulin signaling pathways. The following sections will focus on the physiological impact of such a relationship on glucose homeostasis, insulin secretion and ghrelin levels in cellular, animal and human models.

GHRELIN AND GLUCOSE HOMEOSTASIS

The influence of ghrelin on the regulation of glucose homeostasis was first hypothesized following the observation of a negative correlation between circulating ghrelin and insulin levels in humans[79]. Later, an association between ghrelin and the homeostasis model of assessment, an index of insulin resistance, in women with polycystic ovary syndrome (PCOS) further supported the involvement of ghrelin in the development of insulin resistance and type 2 diabetes[80]. Subsequently, the association of ghrelin with insulin, glucose and insulin resistance indexes was investigated in different populations with definite metabolic profiles. For instance, in obese and non-obese

children and obese adults with or without insulin resistance or type 2 diabetes, pre-meal total ghrelin levels were inversely associated to insulin levels and the severity of insulin resistance[81-83]. The recent development of new and more sensitive immunoassays has allowed the characterization of distinct biological activity of AG and UAG in healthy and pathological conditions. This led to the observation that AG, rather than UAG, reduces insulin secretion while promoting insulin resistance in individuals with or without metabolic dysfunctions[27,84].

Soon after its discovery, ghrelin was shown to be secreted in a pulsatile manner in response to the nutritional status[31]. In clinical studies, ghrelin levels were initially measured from a unique sample in participants submitted to an overnight fast. However more elaborate study designs have been developed to allow the determination of ghrelin levels at different time points in pre-meal and postprandial conditions. The first evidence suggesting the involvement of ghrelin in the regulation of insulin secretion was provided by the observation of a positive association between suppression of total ghrelin levels and insulin concentrations in the postprandial condition in participants with uncomplicated obesity[85]. In addition, total ghrelin levels were negatively correlated to insulin resistance in obese children and adolescents[83].

As previously reviewed[86,87], several research teams have reported a link between ghrelin and the regulation of glucose homeostasis but this was often achieved using one single fasting sample of total ghrelin. Although they provided key information, data generated from these studies were often not in line with results obtained using AG or UAG treatments in cell, animal and human models. Accordingly, the inverse correlations of ghrelin with insulin levels and insulin resistance commonly described in the literature seem rather counter-intuitive at first glance for an adipogenic hormone promoting food intake and decreased energy expenditure. Indeed, we would expect that ghrelin, which drives food intake and adiposity would be positively associated with impaired metabolic functions. It is therefore likely that under physiological conditions, ghrelin acts as a regulator of energy balance to stimulate appetite and the storage of energy substrates while reducing energy expenditure in periods of limited food availability. However, when nutrients are abundant, ghrelin levels decrease to prevent the excessive accumulation of energy substrates. Some also suggest the existence of a state of ghrelin resistance since high-fat consumption blunts the effects of intracerebroventricular-administrated ghrelin on GH secretion, ARC neurons activation and NPY/AgRP expression[88]. From an evolutionary perspective, ghrelin could favor survival for individuals having limited access to nutrients. However, impairments in the regulation of ghrelin secretion, caused by the ingestion of specific nutrients or other genetic/environmental factors, could promote the excessive accumulation of lipids and ultimately the development of metabolic dysfunctions such as insulin resistance and type 2 diabetes.

EFFECTS OF GHRELIN ON INSULIN SECRETION

It was initially reported that a population of ghrelin- and insulin-producing cells would have common embryonic progenitors within the developing endocrine pancreas[89]. In the pancreas, ghrelin-positive ε-cells are found as single cells in islet periphery. Ghrelin is also co-expressed with glucagon-secreting cells in humans and rats[17,90-94]. The expression of GHS-R1a was also detected in islets as well as in several pancreatic cell lines, suggesting that ghrelin and its receptor could influence pancreatic functions in a paracrine manner[95].

As presented in Table 1, the first direct evidence suggesting the influence of ghrelin on the regulation of insulin secretion was provided by Broglio *et al*[21] in healthy volunteers. In fasting condition, AG administered at 1 μg/kg intravenously (*iv*) significantly reduced circulating insulin levels while increasing glycemia. Using the same conditions, AG was shown to reduce insulin secretion in young and elderly participants[106]. Since AG has a relatively short half-life in circulation, continuous administrations of the peptide were performed to confirm the results obtained using bolus injections. The continuous infusion of AG (1 μg/kg per hour) decreased the first phase of insulin secretion postprandially, while causing a significant rise in glycemia[96,107]. This increase in blood glucose was also associated to an enhanced second-phase insulin response. Similarly, Vestergaard *et al*[101-105] observed that AG infusions (0.3 μg/kg per hour to 1.0 μg/kg per hour) promote insulin resistance; however they did not detect any fluctuation in insulin secretion[100,101]. At lower concentrations (0.3 to 1.5 ng/kg per hour), AG infusions reduced insulin secretion and glucose levels[108]. The same authors have also observed a decrease in insulin secretion in response to the administration of physiological concentrations of AG (0.2 and 0.6 ng/kg per hour)[26,109]. Consequently, it is suggested that physiological levels of ghrelin directly impair β-cell functions but the mechanisms underlying these effects remain to be clarified[109]. One appealing hypothesis is that these inhibitory effects of AG on insulin release could be mediated through the stimulation of somatostatin production[97]. In contrast, a single bolus of AG (1 μg/kg) did not induce any alteration of glucose or insulin levels in obese women[110]. In a clinical study, UAG was administered for 16 h at 1.0 μg/kg per hour and the postprandial insulin response was potentiated in healthy volunteers[111]. Following a meal, the inhibitory effect of AG on insulin release was abrogated by the co-administration with UAG[96]. Furthermore, Kiewiet *et al*[112] reported that the combined treatment with AG and UAG increased insulin sensitivity in morbidly obese patients. Altogether, these studies show that ghrelin has complex effects when administered to humans and that the impact of this hormone on glucose homeostasis likely depends on the dose, the nutritional status and the metabolic profile of the population studied. Furthermore, the biphasic insulin response observed

Table 1 Effects of ghrelin treatment in human participants

Model	Treatment	Dose	Condition	Endogenous insulin	Insulin sensitivity
Healthy or hypopituitary humans	AG *vs* Ctrl (*iv*) AG + Arg *vs* Arg (*iv*)	AG: 1 to 2.2 μg/kg Arg: 0.5 g/kg	Fasting (overnight)	Decreased	Decreased[21,96-99]
Healthy or hypopituitary humans	AG + FFA *vs* FFA AG + UAG	AG: 1 μg/kg FFA: 25 g UAG: 1 μg/kg	Fasting (overnight)	Decreased	No change[96,98]
Healthy humans	AG + OGTT (*iv*) *vs* OGTT UAG *vs* Ctrl (*iv*) AG + UAG *vs* Ctrl (*iv*)	AG: 1 μg/kg OGTT: 100g UAG: 1 μg/kg	Fasting (overnight)	No change	No change[96,98]
Healthy humans	AG *vs* Ctrl (*iv*)	AG: 1 μg/kg	Fasting (overnight)	Increased	Decreased[96]
Healthy humans	AG *vs* Ctrl infusion 3h (*iv*)	AG: 5 pmol/kg per minute	Fasting (overnight)	-	Decreased[100]
Healthy, gastrectomized or hypopituitary humans	EHC: AG *vs* Ctrl 5 h (*iv*) pancreatic clamp + EHC:AG *vs* Ctrl 5 h (*iv*)	AG: 5 pmol/kg per minute	Fasting (overnight)	-	Decreased[101-104]
Healthy humans	EHC: AG 5 h (intramuscular)	AG: non-specified supraphysiological dose	Fasting (overnight)	-	Increased[105]

AG: Acylated ghrelin; *iv*: Intravenous; Arg: Arginine; Ctrl: Control; UAG: Unacylated ghrelin; OGTT: Oral glucose tolerance test; EHC: Euglycemic/hyperinsulinemic clamp.

after the administration of AG indicates that the peptide could exert distinct effects on β-cells: an initial inhibition of insulin release combined to a subsequent stimulation of insulin synthesis[96,107]. Further studies are needed to clarify the causes of the variability in insulin secretion and glucose homeostasis observed in response to ghrelin. To do so, it is critical to establish the concentrations at which ghrelin will be administered, and to design clinical protocols with well-established nutritional status and sufficient blood samples to allow detecting positive/negative effects on insulin release under specific metabolic conditions.

Similarly to the available data in humans, data derived from most rodent studies indicate that AG inhibits insulin secretion. In wild type mice, *iv* administrations of AG (5 nmol to 150 nmol) were shown to inhibit fasting and glucose-induced insulin secretion[113]. In contrast, insulinotropic effects have been reported in response to an *iv* injection of AG (25 nmol/L) in rats[114]. In mice, the administration of AG (1 to 10 nmol/kg, *iv*) was also shown to induce biphasic responses[115]. In fact AG was shown to inhibit insulin release by blocking the effects of a cholinergic antagonist on the activation of phospholipase C (PLC) after 2 min but this effect was reversed 6 min after treatment[115]. During the early phase (2 min), ghrelin also promoted the stimulation of insulin secretion by potentiating the response of the phosphodiesterase inhibitor IBMX, but this effect could no longer be observed at 6 min. The same group also reported that the stimulatory effect of ghrelin on insulin release was accompanied by increases in nitric oxide and that this outcome was mediated by the activation of the neuronal constitutive nitric oxide synthase[116]. In mice, AG promptly inhibits insulin release but this effect is reversed over time. This suggests that AG could block the first-phase of insulin secretion and subsequently allow β-cells to release the hormone. Although these effects were modulated through PLC and phosphodiesterase, the mechanisms underlying these observations remain to be elucidated. Consequently, following the description of this biphasic response, it is even possible to speculate that AG's effects could be mediated through the activation of more than one distinct receptor. For instance, these effects could potentially be regulated by the formation of homo- and heterodimers between GHS-R1a and other receptors such as Gpr83 and DRD1/2[37,41]. Interestingly, the expression of both GHS-R1 and DRD2 was previously reported in β-cells[41,95]. Furthermore, DRD2 was shown to inhibit insulin secretion through the activation of the β_2-adrenergic receptor[117]. This indicates that under distinct conditions, AG (and potentially UAG) could mediate the dimerization of GHS-R1 and consequently exert different effects on β-cell functions.

Genetic manipulations have also provided key data regarding ghrelin actions. Overexpression of the ghrelin (*Ghrl*) gene was shown to decrease insulin levels in mice, while its inactivation was shown to enhance insulin secretion and to prevent glucose intolerance[118-120]. In leptin-deficient mice, the deletion of the *Ghrl* gene potentiates insulin secretion and improves glucose homeostasis[121,122]. The pharmacological inhibition of GHS-R1 was also shown to increase insulin secretion and improve glucose homeostasis[123]. In contrast, the ablation of the *Ghs-r1* gene decreased glucose control and reduced insulin secretion in leptin-deficient mice[124]. This impaired insulin response was associated with the upregulation of Uncoupling protein-2 (Ucp-2), Sterol regulatory-element binding protein-1c (Srebp-1c), Carbohydrate-responsive element-binding protein (Chrebp) and Macrophage migration inhibitory factor-1 (Mif-1) and with the downregulation of Hypoxia-inducible factor-1α (Hif-1α), fibroblast growth factor-21 (Fgf-21) and Pancreatic and duodenal homeobox-1 (Pdx-1) in whole pancreases[124]. These genes are known to decrease (*Ucp-2, Srebp-1c*, *Chrebp* and *Mif-1*) or improve (*Hif-1α*, *Fgf-21* and *Pdx-1*) β-cell functions. Another group has also suggested that the effect of AG could be mediated through an increased production of the β-cell autoantigen for type 1 diabetes (IA-2β)[125]. In perfused rat pancreases, the influence of AG on insulin release was also investigated. AG (10 nmol/L) was shown

to promptly decrease insulin *in situ* secretion[126].

The effects of ghrelin on the regulation of insulin secretion were also investigated *in vitro*. In pancreatic tissue fragments of normal and diabetic rats, treatments with AG (1 pmol/L to 1 μmol/L) induced insulinotropic effects[127]. This effect was also observed in response to high doses of AG (0.1 to 1 μmol/L) in cultured isolated mice islets[115]. In contrast, AG was shown to inhibit insulin secretion in immortalized pancreatic β-cells (AG at 0.1 μmol/L) and in cultured mouse islets (AG 1 to 100 pmol/L)[115,128]. It is noteworthy that glucose levels and time of incubation were critical elements mediating AG's effects on insulin release. Accordingly, AG's insulinotropic effects were only detected at glucose concentrations above 8.3 mmol/L[94,115,127,128]. Data obtained in rodents indicate that ghrelin promptly mediates its effects on β-cell function[115]. In the circulation, AG must exert its activity quickly before being degraded. However, *in vitro* AG treatments were carried out for at least 30 min. It is therefore necessary to design experiments allowing the characterization of ghrelin's effects on insulin release in a time-resolved manner. This would allow determining whether ghrelin directly mediates insulin release and/or its synthesis within β-cells.

The effects of AG and UAG on β-cells have been explored to clarify the effects of both ghrelin forms on survival, proliferation and insulin release. It has been demonstrated that both AG and UAG stimulate insulin release in different β-cell lines[129,130]. Furthermore, in response to an intravenous glucose tolerance test, the administration of UAG at 30 nmol/kg was shown to potentiate insulin release in anesthetized rats[131]. Although these effects could not be detected in rat and mouse isolated islets, the inhibitory effect of AG on insulin release was reversed by the combined treatment with UAG[132]. Granata *et al*[130,133] also reported that both ghrelin forms promote cell survival and prevent apoptosis in different β-cell lines. This group also reported that UAG treatment (two subcutaneous administrations of 100 μg/kg for 7 d) could prevent diabetes in newborn rats treated with streptozocin. Although UAG has been shown to influence the release of insulin, important questions remain regarding the mechanisms underlying these effects in the pancreas. For instance, it will be critical to determine whether ghrelin influences the acute release of insulin or its synthesis within β-cells.

The information contained in the above paragraphs suggests that AG inhibits while UAG restores insulin secretion. Although there are many discrepancies in the literature, evidence suggests that the influence of ghrelin on β-cell function depends on the dose of ghrelin used for the treatment as well as the glycemic state under which experiments are carried out. The available data also indicates the relevance of establishing a time-frame during which responses occur. In fact, different groups have described that ghrelin mediates a biphasic response with rapid inhibition and subsequent stimulation of insulin release. Also, homo- and heterodimerization of the GHS-R1a receptor could explain the conflictual observations currently reported in the literature. It is therefore critical to fully determine the (1) optimal doses of AG and UAG; (2) conditions; and (3) the time continuum under which ghrelin influences β-cell functions. Due to its adipogenic nature, it is also of potential interest to investigate whether chronic hyperprolinemia could promote lipotoxicity within β-cells.

EFFECTS OF INSULIN ON CIRCULATING GHRELIN LEVELS

Early after the discovery of ghrelin, an inverse relationship was observed between the ghrelin and insulin levels in animal and human models. In the previous section, the effects of AG and UAG on insulin were reviewed. However, the influence of insulin on both ghrelin forms has also been investigated. It was initially observed that ghrelin levels decrease significantly in healthy participants in response to food intake[134,135]. Moreover, under fasting conditions, ghrelin levels were shown to be inversely correlated with insulin values[79]. Taken together, these elements suggest that insulin could reduce circulating ghrelin levels.

Ghrelin levels have been measured following the intake of different types of meals. However, to isolate the effect of insulin and eliminate potential confounding factors, specific models mimicking postprandial conditions such as the oral glucose tolerance test (OGTT) or the euglycemic hyperinsulinemic clamp (EHC) have been used. It was first reported that total ghrelin levels are significantly reduced in response to OGTT or mixed meals in healthy participants after approximately 35 min[136,137]. In these studies, circulating ghrelin levels were decreased in response to insulin but not following the combined parenteral administration of insulin and glucose[136,137]. These results suggest that decreases in ghrelin levels are not directly mediated by insulin but rather through other mechanisms that require nutrients transiting in the gastrointestinal tract.

Clinical protocols were also designed to study the variations in total ghrelin levels under defined hyperinsulinemic conditions. For instance, in healthy and obese volunteers submitted to EHC or hypoglycemia, total ghrelin levels were significantly reduced[85,138]. Interestingly, in slightly overweight individuals submitted to EHC, total ghrelin concentrations were reduced by 25% and these effects were still detectable 15 min after the insulin infusion ended[139]. Also, under the euglycemic/hyperinsulinemic condition, total ghrelin levels were further reduced by the co-administration with GH and an inhibitor of hormone-sensitive lipase activity in GH-deficient patients[140]. Similar results were observed in response to three-steps hypo-, eu- and hyperglycemic/hyperinsulinemic clamps[141]. Although total ghrelin concentrations were stable before the administration of insulin, the levels of the hormone promptly decreased in response to hyperinsulinemia and remained stable during the hypo- and euglycemic states. However, the most important

reductions in ghrelin levels were noted during the hyperglycemic/hyperinsulinemic conditions. In another study, healthy participants were submitted to three different types of clamps[142]. During the first clamp, hyperglycemia and the resulting elevation of endogenous insulin did not alter ghrelin levels[142,143].

The impact of EHC on ghrelin levels was also studied in different pathological conditions including Pradder-Willi syndrome (PWS), PCOS, and hyper- and hypothyroidism. For instance, elevated total ghrelin levels were reported in children with PWS. The influence of EHC on total ghrelin levels was therefore investigated in both patients with PWS and normal children[144]. Under these conditions, total ghrelin levels were decreased to a greater extent but still remained higher throughout the EHC in patients with PWS compared to controls. Total ghrelin levels were higher in PWS children and their response to EHC was proportional to the one of control individuals. Glucose disposal was similar between normal children and PWS patients, suggesting that under hyperinsulinemic conditions ghrelin levels are reduced in function of the degree of insulin resistance rather than being solely influenced by insulin and glucose levels. To confirm this, patients with type 2 diabetes and healthy individuals were also submitted to EHC. In these patients, fasting total ghrelin levels were lower than in healthy individuals. As expected, total ghrelin levels reduction was significantly less pronounced in patients with type 2 diabetes compared to healthy individuals[145]. This suggests that impairments in IR signaling could disturb the physiological regulation of ghrelin levels. It is recognized that ghrelin levels and insulin sensitivity are lower in women with PCOS. To further study the effect of insulin sensitivity on the regulation of ghrelin levels, women with PCOS were submitted to EHC. Unexpectedly ghrelin levels were not differently modulated in PCOS than in normal women, indicating that the androgen levels could also influence the modulation of ghrelin in this population[146].

Patients with hyperthyroidism also exhibit a negative association between total ghrelin levels and energy expenditure[147]. In these patients, ghrelin levels are also decreased. To investigate the effect of hyperthyroidism normalization, ghrelin levels were measured during EHC before and after medical treatment with antithyroid hormones. Similarly, increased ghrelin levels are observed before and after normalization in patients with hypothyroidism[148]. Despite this difference, ghrelin profiles observed during EHC were not altered by antithyroid treatment or by L-thyroxine (T4) replacement[148,149]. These results indicate that the reduction in ghrelin observed during EHC is independent of thyroid status. The effect of ghrelin on the hypothalamo-pituitary-thyroid axis was also investigated in healthy participants. In contrast to the results obtained in patients who underwent hyper- or hypothyroid normalization, the administration of AG (50 μg) directly increased free T4 while reducing thyroid stimulating hormone concentrations in the circulation[150]. This suggests that the thyroid status does not influence the inhibitory effect of insulin on ghrelin secretion; however ghrelin treatment could directly regulate thyroid functions.

Total ghrelin levels are decreased to a greater extent during EHC in individuals with high insulin sensitivity. However the impact of insulin on the circulating levels of AG and UAG remained uncharacterized for many years. To further characterize the effects of hyperinsulinemia on the different forms of circulating ghrelin, we decided to measure AG and total ghrelin (and estimate UAG levels by subtracting total ghrelin-AG values) during EHC in insulin-sensitive (ISO) and insulin-resistant (IRO) obese postmenopausal women[27]. Total ghrelin and UAG levels were significantly decreased by EHC in ISO and IRO women. However, during EHC, AG levels were significantly reduced only in ISO individuals and the maximal amplitude of reduction was more important than in ISO participants. Similarly, the AG/UAG ratio was significantly lower in ISO women in the fasting condition and throughout EHC. Interestingly, in the total population (ISO + IRO), the maximal amplitude of reduction for total ghrelin and AG were both positively correlated with insulin sensitivity. It was later shown that fasting AG and UAG levels are decreased between the second and the third term of pregnancy in women with diabetes[151]. This was also associated with less important decreases in UAG but not in AG during EHC.

The molecular mechanisms by which insulin regulates ghrelin levels were investigated only in a limited number of studies. Similarly to the results obtained in humans, insulin was shown to reduce total ghrelin levels in rats[152]. Data presented in the signaling section also provided evidence that the gastric insulin signaling activation influences ghrelin mRNA, gastric preproghrelin and circulating ghrelin. Results from two different studies in rodents also indicate that a hyperinsulinemic state could enhance ghrelin mRNA expression but there is no information available on protein levels[31,114]. Although the effects of insulin on total ghrelin levels have been abundantly studied in the literature, it remains that AG and UAG profiles need to be further characterized. Therefore it is critical to decipher the mechanisms mediating the effects of insulin and potential receptor signaling impairments on AG and UAG secretion both in animal and human models under normal and pathological conditions.

CONCLUSION

Although it was discovered more than ten years ago and was the object of an impressive number of publications, important questions still remain regarding the physiological control of AG and UAG secretion and the distinct role of both ghrelin forms in the regulation of metabolic functions. The present work intends to highlight the interrelationships between ghrelin, insulin and glucose homeostasis. Available data indicate that ghrelin influences insulin secretion and vice versa. New evidence suggests the existence of crosstalks between the signaling pathways induced by the activation of the native ghrelin receptor, GHS-R1a and the insulin receptor. However, these interactions seem to oppose themselves

as they are taking place in the central nervous system or in the periphery. This suggests that in different tissues and organs, the heterodimerization of GHS-R1a with Gpr83, DRD1/2, MC3R and potentially other receptors could trigger the activation of distinct signaling pathways. Other important issues were denoted in the literature regarding the insulinotropic effects of ghrelin in cellular, animal and human models. This suggests the critical need to better determine doses under which AG and UAG optimally activate distinct metabolic functions. Taking into consideration the complexity of ghrelin's physiology it is also important to characterize the conditions under which altered responses to AG and UAG are observed. Overall, these clarifications should provide a better understanding of the mechanisms underlying AG and UAG secretion as well as to allow the deciphering of their role in the regulation of distinct metabolic functions.

REFERENCES

1 **Olshansky SJ**, Passaro DJ, Hershow RC, Layden J, Carnes BA, Brody J, Hayflick L, Butler RN, Allison DB, Ludwig DS. A potential decline in life expectancy in the United States in the 21st century. *N Engl J Med* 2005; **352**: 1138-1145 [PMID: 15784668 DOI: 10.1056/NEJMsr043743]

2 **Ou HY**, Wang CY, Yang YC, Chen MF, Chang CJ. The association between nonalcoholic fatty pancreas disease and diabetes. *PLoS One* 2013; **8**: e62561 [PMID: 23671610 DOI: 10.1371/journal.pone.0062561]

3 **Meikle PJ**, Christopher MJ. Lipidomics is providing new insight into the metabolic syndrome and its sequelae. *Curr Opin Lipidol* 2011; **22**: 210-215 [PMID: 21378565 DOI: 10.1097/MOL.0b013e3283453dbe]

4 **Mlinar B**, Marc J. New insights into adipose tissue dysfunction in insulin resistance. *Clin Chem Lab Med* 2011; **49**: 1925-1935 [PMID: 21892913 DOI: 10.1515/CCLM.2011.697]

5 **Delzenne NM**, Neyrinck AM, Bäckhed 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]

6 **Piya MK**, McTernan PG, Kumar S. Adipokine inflammation and insulin resistance: the role of glucose, lipids and endotoxin. *J Endocrinol* 2013; **216**: T1-T15 [PMID: 23160966 DOI: 10.1530/JOE-12-0498]

7 **Pacifico L**, Di Martino M, De Merulis A, Bezzi M, Osborn JF, Catalano C, Chiesa C. Left ventricular dysfunction in obese children and adolescents with nonalcoholic fatty liver disease. *Hepatology* 2014; **59**: 461-470 [PMID: 23843206 DOI: 10.1002/hep.26610]

8 **Miegueu P**, Cianflone K, Richard D, St-Pierre DH. Motilin stimulates preadipocyte proliferation and differentiation and adipocyte lipid storage. *Am J Physiol Endocrinol Metab* 2011; **301**: E758-E766 [PMID: 21771971 DOI: 10.1152/ajpendo.00089.2011]

9 **Miegueu P**, Cianflone K, Richard D, St-Pierre DH. Effect of secretin on preadipocyte, differentiating and mature adipocyte functions. *Int J Obes* (Lond) 2013; **37**: 366-374 [PMID: 22565418 DOI: 10.1038/ijo.2012.73]

10 **Miegueu P**, St Pierre D, Broglio F, Cianflone K. Effect of desacyl ghrelin, obestatin and related peptides on triglyceride storage, metabolism and GHSR signaling in 3T3-L1 adipocytes. *J Cell Biochem* 2011; **112**: 704-714 [PMID: 21268092 DOI: 10.1002/jcb.22983]

11 **Neary MT**, Batterham RL. Gut hormones: implications for the treatment of obesity. *Pharmacol Ther* 2009; **124**: 44-56 [PMID: 19560488 DOI: 10.1016/j.pharmthera.2009.06.005]

12 **Boguszewski CL**, Paz-Filho G, Velloso LA. Neuroendocrine body weight regulation: integration between fat tissue, gastrointestinal tract, and the brain. *Endokrynol Pol* 2010; **61**: 194-206 [PMID: 20464707]

13 **Kojima M**, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 1999; **402**: 656-660 [PMID: 10604470 DOI: 10.1038/45230]

14 **Ariyasu H**, Takaya K, Tagami T, Ogawa Y, Hosoda K, Akamizu T, Suda M, Koh T, Natsui K, Toyooka S, Shirakami G, Usui T, Shimatsu A, Doi K, Hosoda H, Kojima M, Kangawa K, Nakao K. Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. *J Clin Endocrinol Metab* 2001; **86**: 4753-4758 [PMID: 11600536]

15 **Hosoda H**, Kojima M, Matsuo H, Kangawa K. Ghrelin and des-acyl ghrelin: two major forms of rat ghrelin peptide in gastrointestinal tissue. *Biochem Biophys Res Commun* 2000; **279**: 909-913 [PMID: 11162448 DOI: 10.1006/bbrc.2000.4039]

16 **Ghelardoni S**, Carnicelli V, Frascarelli S, Ronca-Testoni S, Zucchi R. Ghrelin tissue distribution: comparison between gene and protein expression. *J Endocrinol Invest* 2006; **29**: 115-121 [PMID: 16610236]

17 **Raghay K**, Gallego R, Scoazec JY, Garcia-Caballero T, Morel G. Different ghrelin localisation in adult human and rat endocrine pancreas. *Cell Tissue Res* 2013; **352**: 487-494 [PMID: 23584608 DOI: 10.1007/s00441-013-1593-y]

18 **Date Y**, Kojima M, Hosoda H, Sawaguchi A, Mondal MS, Suganuma T, Matsukura S, Kangawa K, Nakazato M. Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. *Endocrinology* 2000; **141**: 4255-4261 [PMID: 11089560]

19 **Takaya K**, Ariyasu H, Kanamoto N, Iwakura H, Yoshimoto A, Harada M, Mori K, Komatsu Y, Usui T, Shimatsu A, Ogawa Y, Hosoda K, Akamizu T, Kojima M, Kangawa K, Nakao K. Ghrelin strongly stimulates growth hormone release in humans. *J Clin Endocrinol Metab* 2000; **85**: 4908-4911 [PMID: 11134161]

20 **Arvat E**, Di Vito L, Broglio F, Papotti M, Muccioli G, Dieguez C, Casanueva FF, Deghenghi R, Camanni F, Ghigo E. Preliminary evidence that Ghrelin, the natural GH secretagogue (GHS)-receptor ligand, strongly stimulates GH secretion in humans. *J Endocrinol Invest* 2000; **23**: 493-495 [PMID: 11021763]

21 **Broglio F**, Arvat E, Benso A, Gottero C, Muccioli G, Papotti M, van der Lely AJ, Deghenghi R, Ghigo E. Ghrelin, a natural GH secretagogue produced by the stomach, induces hyperglycemia and reduces insulin secretion in humans. *J Clin Endocrinol Metab* 2001; **86**: 5083-5086 [PMID: 11600590]

22 **Kirchner H**, Heppner KM, Tschöp MH. The role of ghrelin in the control of energy balance. *Handb Exp Pharmacol* 2012; (**209**): 161-184 [PMID: 22249814 DOI: 10.1007/978-3-642-24716-3_7]

23 **Kaiya H**, Kangawa K, Miyazato M. What is the general action of ghrelin for vertebrates? - comparisons of ghrelin's effects across vertebrates. *Gen Comp Endocrinol* 2013; **181**: 187-191 [PMID: 23178701 DOI: 10.1016/j.ygcen.2012.10.015]

24 **Ukkola O**. Ghrelin in Type 2 diabetes mellitus and metabolic syndrome. *Mol Cell Endocrinol* 2011; **340**: 26-28 [PMID: 21419192 DOI: 10.1016/j.mce.2011.02.009]

25 **Yang J**, Brown MS, Liang G, Grishin NV, Goldstein JL. Identification of the acyltransferase that octanoylates ghrelin, an appetite-stimulating peptide hormone. *Cell* 2008; **132**: 387-396 [PMID: 18267071 DOI: 10.1016/j.cell.2008.01.017]

26 **Tong J**, Dave N, Mugundu GM, Davis HW, Gaylinn BD, Thorner MO, Tschöp MH, D'Alessio D, Desai PB. The pharmacokinetics of acyl, des-acyl, and total ghrelin in healthy human subjects. *Eur J Endocrinol* 2013; **168**: 821-828 [PMID:

23482590 DOI: 10.1530/EJE-13-0072]
27 **St-Pierre DH**, Karelis AD, Coderre L, Malita F, Fontaine J, Mignault D, Brochu M, Bastard JP, Cianflone K, Doucet E, Imbeault P, Rabasa-Lhoret R. Association of acylated and nonacylated ghrelin with insulin sensitivity in overweight and obese postmenopausal women. *J Clin Endocrinol Metab* 2007; **92**: 264-269 [PMID: 17062757 DOI: 10.1210/jc.2006-1603]
28 **Howard AD**, Feighner SD, Cully DF, Arena JP, Liberator PA, Rosenblum CI, Hamelin M, Hreniuk DL, Palyha OC, Anderson J, Paress PS, Diaz C, Chou M, Liu KK, McKee KK, Pong SS, Chaung LY, Elbrecht A, Dashkevicz M, Heavens R, Rigby M, Sirinathsinghji DJ, Dean DC, Melillo DG, Patchett AA, Nargund R, Griffin PR, DeMartino JA, Gupta SK, Schaeffer JM, Smith RG, Van der Ploeg LH. A receptor in pituitary and hypothalamus that functions in growth hormone release. *Science* 1996; **273**: 974-977 [PMID: 8688086]
29 **Wang L**, Saint-Pierre DH, Taché Y. Peripheral ghrelin selectively increases Fos expression in neuropeptide Y - synthesizing neurons in mouse hypothalamic arcuate nucleus. *Neurosci Lett* 2002; **325**: 47-51 [PMID: 12023064]
30 **Tschöp M**, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. *Nature* 2000; **407**: 908-913 [PMID: 11057670 DOI: 10.1038/35038090]
31 **Toshinai K**, Mondal MS, Nakazato M, Date Y, Murakami N, Kojima M, Kangawa K, Matsukura S. Upregulation of Ghrelin expression in the stomach upon fasting, insulin-induced hypoglycemia, and leptin administration. *Biochem Biophys Res Commun* 2001; **281**: 1220-1225 [PMID: 11243865 DOI: 10.1006/bbrc.2001.4518]
32 **Halem HA**, Taylor JE, Dong JZ, Shen Y, Datta R, Abizaid A, Diano S, Horvath T, Zizzari P, Bluet-Pajot MT, Epelbaum J, Culler MD. Novel analogs of ghrelin: physiological and clinical implications. *Eur J Endocrinol* 2004; **151** Suppl 1: S71-S75 [PMID: 15339248]
33 **Halem HA**, Taylor JE, Dong JZ, Shen Y, Datta R, Abizaid A, Diano S, Horvath TL, Culler MD. A novel growth hormone secretagogue-1a receptor antagonist that blocks ghrelin-induced growth hormone secretion but induces increased body weight gain. *Neuroendocrinology* 2005; **81**: 339-349 [PMID: 16210868 DOI: 10.1159/000088796]
34 **Bluet-Pajot MT**, Epelbaum J, Gourdji D, Hammond C, Kordon C. Hypothalamic and hypophyseal regulation of growth hormone secretion. *Cell Mol Neurobiol* 1998; **18**: 101-123 [PMID: 9524732]
35 **Sun Y**, Wang P, Zheng H, Smith RG. Ghrelin stimulation of growth hormone release and appetite is mediated through the growth hormone secretagogue receptor. *Proc Natl Acad Sci USA* 2004; **101**: 4679-4684 [PMID: 15070777 DOI: 10.1073/pnas.0305930101]
36 **Castañeda TR**, Tong J, Datta R, Culler M, Tschöp MH. Ghrelin in the regulation of body weight and metabolism. *Front Neuroendocrinol* 2010; **31**: 44-60 [PMID: 19896496 DOI: 10.1016/j.yfrne.2009.10.008]
37 **Müller TD**, Müller A, Yi CX, Habegger KM, Meyer CW, Gaylinn BD, Finan B, Heppner K, Trivedi C, Bielohuby M, Abplanalp W, Meyer F, Piechowski CL, Pratzka J, Stemmer K, Holland J, Hembree J, Bhardwaj N, Raver C, Ottaway N, Krishna R, Sah R, Sallee FR, Woods SC, Perez-Tilve D, Bidlingmaier M, Thorner MO, Krude H, Smiley D, DiMarchi R, Hofmann S, Pfluger PT, Kleinau G, Biebermann H, Tschöp MH. The orphan receptor Gpr83 regulates systemic energy metabolism via ghrelin-dependent and ghrelin-independent mechanisms. *Nat Commun*2013; **4**: 1968 [PMID: 23744028 DOI: 10.1038/ncomms2968]
38 **Schellekens H**, Dinan TG, Cryan JF. Taking two to tango: a role for ghrelin receptor heterodimerization in stress and reward. *Front Neurosci* 2013; **7**: 148 [PMID: 24009547 DOI: 10.3389/fnins.2013.00148]
39 **Mary S**, Fehrentz JA, Damian M, Gaibelet G, Orcel H, Verdié P, Mouillac B, Martinez J, Marie J, Banères JL. Heterodimerization with Its splice variant blocks the ghrelin receptor 1a in a non-signaling conformation: a study with a purified heterodimer assembled into lipid discs. *J Biol Chem* 2013; **288**: 24656-24665 [PMID: 23839942 DOI: 10.1074/jbc.M113.453423]
40 **Schellekens H**, van Oeffelen WE, Dinan TG, Cryan JF. Promiscuous dimerization of the growth hormone secretagogue receptor (GHS-R1a) attenuates ghrelin-mediated signaling. *J Biol Chem* 2013; **288**: 181-191 [PMID: 23161547 DOI: 10.1074/jbc.M112.382473]
41 **Kern A**, Albarran-Zeckler R, Walsh HE, Smith RG. Apoghrelin receptor forms heteromers with DRD2 in hypothalamic neurons and is essential for anorexigenic effects of DRD2 agonism. *Neuron* 2012; **73**: 317-332 [PMID: 22284186 DOI: 10.1016/j.neuron.2011.10.038]
42 **Willesen MG**, Kristensen P, Rømer J. Co-localization of growth hormone secretagogue receptor and NPY mRNA in the arcuate nucleus of the rat. *Neuroendocrinology* 1999; **70**: 306-316 [PMID: 10567856]
43 **Kohno D**, Gao HZ, Muroya S, Kikuyama S, Yada T. Ghrelin directly interacts with neuropeptide-Y-containing neurons in the rat arcuate nucleus: Ca2+ signaling via protein kinase A and N-type channel-dependent mechanisms and cross-talk with leptin and orexin. *Diabetes* 2003; **52**: 948-956 [PMID: 12663466]
44 **Kamegai J**, Tamura H, Shimizu T, Ishii S, Sugihara H, Wakabayashi I. Central effect of ghrelin, an endogenous growth hormone secretagogue, on hypothalamic peptide gene expression. *Endocrinology* 2000; **141**: 4797-4800 [PMID: 11108296]
45 **Hewson AK**, Dickson SL. Systemic administration of ghrelin induces Fos and Egr-1 proteins in the hypothalamic arcuate nucleus of fasted and fed rats. *J Neuroendocrinol* 2000; **12**: 1047-1049 [PMID: 11069119]
46 **Lawrence CB**, Snape AC, Baudoin FM, Luckman SM. Acute central ghrelin and GH secretagogues induce feeding and activate brain appetite centers. *Endocrinology* 2002; **143**: 155-162 [PMID: 11751604]
47 **Ishii S**, Kamegai J, Tamura H, Shimizu T, Sugihara H, Oikawa S. Hypothalamic neuropeptide Y/Y1 receptor pathway activated by a reduction in circulating leptin, but not by an increase in circulating ghrelin, contributes to hyperphagia associated with triiodothyronine-induced thyrotoxicosis. *Neuroendocrinology* 2003; **78**: 321-330 [PMID: 14688445]
48 **Kamegai J**, Tamura H, Shimizu T, Ishii S, Sugihara H, Oikawa S. Regulation of the ghrelin gene: growth hormone-releasing hormone upregulates ghrelin mRNA in the pituitary. *Endocrinology* 2001; **142**: 4154-4157 [PMID: 11517195]
49 **Shintani M**, Ogawa Y, Ebihara K, Aizawa-Abe M, Miyanaga F, Takaya K, Hayashi T, Inoue G, Hosoda K, Kojima M, Kangawa K, Nakao K. Ghrelin, an endogenous growth hormone secretagogue, is a novel orexigenic peptide that antagonizes leptin action through the activation of hypothalamic neuropeptide Y/Y1 receptor pathway. *Diabetes* 2001; **50**: 227-232 [PMID: 11272130]
50 **Heppner KM**, Piechowski CL, Müller A, Ottaway N, Sisley S, Smiley DL, Habegger KM, Pfluger PT, Dimarchi R, Biebermann H, Tschöp MH, Sandoval DA, Perez-Tilve D. Both acyl and des-acyl ghrelin regulate adiposity and glucose metabolism via central nervous system ghrelin receptors. *Diabetes* 2014; **63**: 122-131 [PMID: 24062249 DOI: 10.2337/db13-0414]
51 **Zigman JM**, Nakano Y, Coppari R, Balthasar N, Marcus JN, Lee CE, Jones JE, Deysher AE, Waxman AR, White RD, Williams TD, Lachey JL, Seeley RJ, Lowell BB, Elmquist JK. Mice lacking ghrelin receptors resist the development of diet-induced obesity. *J Clin Invest* 2005; **115**: 3564-3572 [PMID: 16322794 DOI: 10.1172/JCI26002]
52 **Kamegai J**, Tamura H, Shimizu T, Ishii S, Sugihara H, Wakabayashi I. Chronic central infusion of ghrelin increases hypothalamic neuropeptide Y and Agouti-related protein

mRNA levels and body weight in rats. *Diabetes* 2001; **50**: 2438-2443 [PMID: 11679419]
53 **Kwon Jeong J**, Dae Kim J, Diano S. Ghrelin regulates hypothalamic prolyl carboxypeptidase expression in mice. *Mol Metab* 2013; **2**: 23-30 [PMID: 24024131 DOI: 10.1016/j.molmet.2013.01.002]
54 **Fan W**, Dinulescu DM, Butler AA, Zhou J, Marks DL, Cone RD. The central melanocortin system can directly regulate serum insulin levels. *Endocrinology* 2000; **141**: 3072-3079 [PMID: 10965876]
55 **Benoit SC**, Air EL, Coolen LM, Strauss R, Jackman A, Clegg DJ, Seeley RJ, Woods SC. The catabolic action of insulin in the brain is mediated by melanocortins. *J Neurosci* 2002; **22**: 9048-9052 [PMID: 12388611]
56 **Seoane LM**, López M, Tovar S, Casanueva FF, Señarís R, Diéguez C. Agouti-related peptide, neuropeptide Y, and somatostatin-producing neurons are targets for ghrelin actions in the rat hypothalamus. *Endocrinology* 2003; **144**: 544-551 [PMID: 12538615]
57 **Smith RG**. Development of growth hormone secretagogues. *Endocr Rev* 2005; **26**: 346-360 [PMID: 15814848 DOI: 10.1210/er.2004-0019]
58 **de Lartigue G**, Dimaline R, Varro A, Dockray GJ. Cocaine- and amphetamine-regulated transcript: stimulation of expression in rat vagal afferent neurons by cholecystokinin and suppression by ghrelin. *J Neurosci* 2007; **27**: 2876-2882 [PMID: 17360909 DOI: 10.1523/JNEUROSCI.5508-06.2007]
59 **Chen C**, Wu D, Clarke IJ. Signal transduction systems employed by synthetic GH-releasing peptides in somatotrophs. *J Endocrinol* 1996; **148**: 381-386 [PMID: 8778215]
60 **Hardie DG**, Ross FA, Hawley SA. AMPK: a nutrient and energy sensor that maintains energy homeostasis. *Nat Rev Mol Cell Biol* 2012; **13**: 251-262 [PMID: 22436748 DOI: 10.1038/nrm3311]
61 **Kohno D**, Sone H, Minokoshi Y, Yada T. Ghrelin raises [Ca2+]i via AMPK in hypothalamic arcuate nucleus NPY neurons. *Biochem Biophys Res Commun* 2008; **366**: 388-392 [PMID: 18068666 DOI: 10.1016/j.bbrc.2007.11.166]
62 **Andersson U**, Filipsson K, Abbott CR, Woods A, Smith K, Bloom SR, Carling D, Small CJ. AMP-activated protein kinase plays a role in the control of food intake. *J Biol Chem* 2004; **279**: 12005-12008 [PMID: 14742438 DOI: 10.1074/jbc.C300557200]
63 **López M**, Lage R, Saha AK, Pérez-Tilve D, Vázquez MJ, Varela L, Sangiao-Alvarellos S, Tovar S, Raghay K, Rodríguez-Cuenca S, Deoliveira RM, Castañeda T, Datta R, Dong JZ, Culler M, Sleeman MW, Alvarez CV, Gallego R, Lelliott CJ, Carling D, Tschöp MH, Diéguez C, Vidal-Puig A. Hypothalamic fatty acid metabolism mediates the orexigenic action of ghrelin. *Cell Metab* 2008; **7**: 389-399 [PMID: 18460330 DOI: 10.1016/j.cmet.2008.03.006]
64 **Laplante M**, Sabatini DM. mTOR signaling at a glance. *J Cell Sci* 2009; **122**: 3589-3594 [PMID: 19812304 DOI: 10.1242/jcs.051011]
65 **Laplante M**, Sabatini DM. mTOR signaling in growth control and disease. *Cell* 2012; **149**: 274-293 [PMID: 22500797 DOI: 10.1016/j.cell.2012.03.017]
66 **Blouet C**, Ono H, Schwartz GJ. Mediobasal hypothalamic p70 S6 kinase 1 modulates the control of energy homeostasis. *Cell Metab* 2008; **8**: 459-467 [PMID: 19041762 DOI: 10.1016/j.cmet.2008.10.004]
67 **Cota D**, Matter EK, Woods SC, Seeley RJ. The role of hypothalamic mammalian target of rapamycin complex 1 signaling in diet-induced obesity. *J Neurosci* 2008; **28**: 7202-7208 [PMID: 18614690 DOI: 10.1523/JNEUROSCI.1389-08.2008]
68 **Martins L**, Fernández-Mallo D, Novelle MG, Vázquez MJ, Tena-Sempere M, Nogueiras R, López M, Diéguez C. Hypothalamic mTOR signaling mediates the orexigenic action of ghrelin. *PLoS One* 2012; **7**: e46923 [PMID: 23056530 DOI: 10.1371/journal.pone.0046923]
69 **Minokoshi Y**, Alquier T, Furukawa N, Kim YB, Lee A, Xue B, Mu J, Foufelle F, Ferré P, Birnbaum MJ, Stuck BJ, Kahn BB. AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. *Nature* 2004; **428**: 569-574 [PMID: 15058305 DOI: 10.1038/nature02440]
70 **Sangiao-Alvarellos S**, Varela L, Vázquez MJ, Da Boit K, Saha AK, Cordido F, Diéguez C, López M. Influence of ghrelin and growth hormone deficiency on AMP-activated protein kinase and hypothalamic lipid metabolism. *J Neuroendocrinol* 2010; **22**: 543-556 [PMID: 20298456 DOI: 10.1111/j.1365-2826.2010.01994.x]
71 **Choi K**, Roh SG, Hong YH, Shrestha YB, Hishikawa D, Chen C, Kojima M, Kangawa K, Sasaki S. The role of ghrelin and growth hormone secretagogues receptor on rat adipogenesis. *Endocrinology* 2003; **144**: 754-759 [PMID: 12586750]
72 **Barazzoni R**, Bosutti A, Stebel M, Cattin MR, Roder E, Visintin L, Cattin L, Biolo G, Zanetti M, Guarnieri G. Ghrelin regulates mitochondrial-lipid metabolism gene expression and tissue fat distribution in liver and skeletal muscle. *Am J Physiol Endocrinol Metab* 2005; **288**: E228-E235 [PMID: 15328073 DOI: 10.1152/ajpendo.00115.2004]
73 **Laplante M**, Sabatini DM. An emerging role of mTOR in lipid biosynthesis. *Curr Biol* 2009; **19**: R1046-R1052 [PMID: 19948145 DOI: 10.1016/j.cub.2009.09.058]
74 **Demers A**, Caron V, Rodrigue-Way A, Wahli W, Ong H, Tremblay A. A concerted kinase interplay identifies PPARgamma as a molecular target of ghrelin signaling in macrophages. *PLoS One* 2009; **4**: e7728 [PMID: 19888469 DOI: 10.1371/journal.pone.0007728]
75 **Dagon Y**, Avraham Y, Berry EM. AMPK activation regulates apoptosis, adipogenesis, and lipolysis by eIF2alpha in adipocytes. *Biochem Biophys Res Commun* 2006; **340**: 43-47 [PMID: 16377306 DOI: 10.1016/j.bbrc.2005.11.159]
76 **Vingtdeux V**, Chandakkar P, Zhao H, Davies P, Marambaud P. Small-molecule activators of AMP-activated protein kinase (AMPK), RSVA314 and RSVA405, inhibit adipogenesis. *Mol Med* 2011; **17**: 1022-1030 [PMID: 21647536 DOI: 10.2119/molmed.2011.00163]
77 **Xu G**, Li Y, An W, Zhao J, Xiang X, Ding L, Li Z, Guan Y, Wang X, Tang C, Zhu Y, Wang N, Li X, Mulholland M, Zhang W. Regulation of gastric hormones by systemic rapamycin. *Peptides* 2010; **31**: 2185-2192 [PMID: 20804797 DOI: 10.1016/j.peptides.2010.08.018]
78 **Xu G**, Li Y, An W, Li S, Guan Y, Wang N, Tang C, Wang X, Zhu Y, Li X, Mulholland MW, Zhang W. Gastric mammalian target of rapamycin signaling regulates ghrelin production and food intake. *Endocrinology* 2009; **150**: 3637-3644 [PMID: 19406939 DOI: 10.1210/en.2009-0372]
79 **Tschöp M**, Weyer C, Tataranni PA, Devanarayan V, Ravussin E, Heiman ML. Circulating ghrelin levels are decreased in human obesity. *Diabetes* 2001; **50**: 707-709 [PMID: 11289032]
80 **Schöfl C**, Horn R, Schill T, Schlösser HW, Müller MJ, Brabant G. Circulating ghrelin levels in patients with polycystic ovary syndrome. *J Clin Endocrinol Metab* 2002; **87**: 4607-4610 [PMID: 12364442]
81 **Katsuki A**, Urakawa H, Gabazza EC, Murashima S, Nakatani K, Togashi K, Yano Y, Adachi Y, Sumida Y. Circulating levels of active ghrelin is associated with abdominal adiposity, hyperinsulinemia and insulin resistance in patients with type 2 diabetes mellitus. *Eur J Endocrinol* 2004; **151**: 573-577 [PMID: 15538935]
82 **McLaughlin T**, Abbasi F, Lamendola C, Frayo RS, Cummings DE. Plasma ghrelin concentrations are decreased in insulin-resistant obese adults relative to equally obese insulin-sensitive controls. *J Clin Endocrinol Metab* 2004; **89**: 1630-1635 [PMID: 15070922]
83 **Ikezaki A**, Hosoda H, Ito K, Iwama S, Miura N, Matsuoka H, Kondo C, Kojima M, Kangawa K, Sugihara S. Fasting plasma ghrelin levels are negatively correlated with insulin resis-

tance and PAI-1, but not with leptin, in obese children and adolescents. *Diabetes* 2002; **51**: 3408-3411 [PMID: 12453893]
84 **Barazzoni R**, Zanetti M, Ferreira C, Vinci P, Pirulli A, Mucci M, Dore F, Fonda M, Ciocchi B, Cattin L, Guarnieri G. Relationships between desacylated and acylated ghrelin and insulin sensitivity in the metabolic syndrome. *J Clin Endocrinol Metab* 2007; **92**: 3935-3940 [PMID: 17652221 DOI: 10.1210/jc.2006-2527]
85 **Leonetti F**, Iacobellis G, Ribaudo MC, Zappaterreno A, Tiberti C, Iannucci CV, Vecci E, Di Mario U. Acute insulin infusion decreases plasma ghrelin levels in uncomplicated obesity. *Regul Pept* 2004; **122**: 179-183 [PMID: 15491789 DOI: 10.1016/j.regpep.2004.06.014]
86 **Delhanty PJ**, van der Lely AJ. Ghrelin and glucose homeostasis. *Peptides* 2011; **32**: 2309-2318 [PMID: 21396419 DOI: 10.1016/j.peptides.2011.03.001]
87 **Briggs DI**, Andrews ZB. A recent update on the role of ghrelin in glucose homeostasis. *Curr Diabetes Rev* 2011; **7**: 201-207 [PMID: 21539509]
88 **Briggs DI**, Enriori PJ, Lemus MB, Cowley MA, Andrews ZB. Diet-induced obesity causes ghrelin resistance in arcuate NPY/AgRP neurons. *Endocrinology* 2010; **151**: 4745-4755 [PMID: 20826561 DOI: 10.1210/en.2010-0556]
89 **Prado CL**, Pugh-Bernard AE, Elghazi L, Sosa-Pineda B, Sussel L. Ghrelin cells replace insulin-producing beta cells in two mouse models of pancreas development. *Proc Natl Acad Sci USA* 2004; **101**: 2924-2929 [PMID: 14970313 DOI: 10.1073/pnas.0308604100]
90 **Wierup N**, Svensson H, Mulder H, Sundler F. The ghrelin cell: a novel developmentally regulated islet cell in the human pancreas. *Regul Pept* 2002; **107**: 63-69 [PMID: 12137967]
91 **Kageyama H**, Funahashi H, Hirayama M, Takenoya F, Kita T, Kato S, Sakurai J, Lee EY, Inoue S, Date Y, Nakazato M, Kangawa K, Shioda S. Morphological analysis of ghrelin and its receptor distribution in the rat pancreas. *Regul Pept* 2005; **126**: 67-71 [PMID: 15620416 DOI: 10.1016/j.regpep.2004.08.031]
92 **Muccioli G**, Tschöp M, Papotti M, Deghenghi R, Heiman M, Ghigo E. Neuroendocrine and peripheral activities of ghrelin: implications in metabolism and obesity. *Eur J Pharmacol* 2002; **440**: 235-254 [PMID: 12007539]
93 **Gnanapavan S**, Kola B, Bustin SA, Morris DG, McGee P, Fairclough P, Bhattacharya S, Carpenter R, Grossman AB, Korbonits M. The tissue distribution of the mRNA of ghrelin and subtypes of its receptor, GHS-R, in humans. *J Clin Endocrinol Metab* 2002; **87**: 2988 [PMID: 12050285]
94 **Date Y**, Nakazato M, Hashiguchi S, Dezaki K, Mondal MS, Hosoda H, Kojima M, Kangawa K, Arima T, Matsuo H, Yada T, Matsukura S. Ghrelin is present in pancreatic alpha-cells of humans and rats and stimulates insulin secretion. *Diabetes* 2002; **51**: 124-129 [PMID: 11756331]
95 **Nakashima K**, Kanda Y, Hirokawa Y, Kawasaki F, Matsuki M, Kaku K. MIN6 is not a pure beta cell line but a mixed cell line with other pancreatic endocrine hormones. *Endocr J* 2009; **56**: 45-53 [PMID: 18845907]
96 **Gauna C**, Meyler FM, Janssen JA, Delhanty PJ, Abribat T, van Koetsveld P, Hofland LJ, Broglio F, Ghigo E, van der Lely AJ. Administration of acylated ghrelin reduces insulin sensitivity, whereas the combination of acylated plus unacylated ghrelin strongly improves insulin sensitivity. *J Clin Endocrinol Metab* 2004; **89**: 5035-5042 [PMID: 15472202 DOI: 10.1210/jc.2004-0363]
97 **Arosio M**, Ronchi CL, Gebbia C, Cappiello V, Beck-Peccoz P, Peracchi M. Stimulatory effects of ghrelin on circulating somatostatin and pancreatic polypeptide levels. *J Clin Endocrinol Metab* 2003; **88**: 701-704 [PMID: 12574202]
98 **Broglio F**, Gottero C, Benso A, Prodam F, Destefanis S, Gauna C, Maccario M, Deghenghi R, van der Lely AJ, Ghigo E. Effects of ghrelin on the insulin and glycemic responses to glucose, arginine, or free fatty acids load in humans. *J Clin Endocrinol Metab* 2003; **88**: 4268-4272 [PMID: 12970297]
99 **Broglio F**, Gottero C, Prodam F, Gauna C, Muccioli G, Papotti M, Abribat T, Van Der Lely AJ, Ghigo E. Non-acylated ghrelin counteracts the metabolic but not the neuroendocrine response to acylated ghrelin in humans. *J Clin Endocrinol Metab* 2004; **89**: 3062-3065 [PMID: 15181099 DOI: 10.1210/jc.2003-031964]
100 **Vestergaard ET**, Hansen TK, Gormsen LC, Jakobsen P, Moller N, Christiansen JS, Jorgensen JO. Constant intravenous ghrelin infusion in healthy young men: clinical pharmacokinetics and metabolic effects. *Am J Physiol Endocrinol Metab* 2007; **292**: E1829-E1836 [PMID: 17311892 DOI: 10.1152/ajpendo.00682.2006]
101 **Vestergaard ET**, Djurhuus CB, Gjedsted J, Nielsen S, Møller N, Holst JJ, Jørgensen JO, Schmitz O. Acute effects of ghrelin administration on glucose and lipid metabolism. *J Clin Endocrinol Metab* 2008; **93**: 438-444 [PMID: 18042651 DOI: 10.1210/jc.2007-2018]
102 **Vestergaard ET**, Gormsen LC, Jessen N, Lund S, Hansen TK, Moller N, Jorgensen JO. Ghrelin infusion in humans induces acute insulin resistance and lipolysis independent of growth hormone signaling. *Diabetes* 2008; **57**: 3205-3210 [PMID: 18776138 DOI: 10.2337/db08-0025]
103 **Vestergaard ET**, Krag MB, Poulsen MM, Pedersen SB, Moller N, Jorgensen JO, Jessen N. Ghrelin- and GH-induced insulin resistance: no association with retinol-binding protein-4. *Endocr Connect* 2013; **2**: 96-103 [PMID: 23781325 DOI: 10.1530/EC-13-0019]
104 **Damjanovic SS**, Lalic NM, Pesko PM, Petakov MS, Jotic A, Miljic D, Lalic KS, Lukic L, Djurovic M, Djukic VB. Acute effects of ghrelin on insulin secretion and glucose disposal rate in gastrectomized patients. *J Clin Endocrinol Metab* 2006; **91**: 2574-2581 [PMID: 16621911 DOI: 10.1210/jc.2005-1482]
105 **Vestergaard ET**, Møller N, Jørgensen JO. Acute peripheral tissue effects of ghrelin on interstitial levels of glucose, glycerol, and lactate: a microdialysis study in healthy human subjects. *Am J Physiol Endocrinol Metab* 2013; **304**: E1273-E1280 [PMID: 23592479 DOI: 10.1152/ajpendo.00662.2012]
106 **Broglio F**, Benso A, Castiglioni C, Gottero C, Prodam F, Destefanis S, Gauna C, van der Lely AJ, Deghenghi R, Bo M, Arvat E, Ghigo E. The endocrine response to ghrelin as a function of gender in humans in young and elderly subjects. *J Clin Endocrinol Metab* 2003; **88**: 1537-1542 [PMID: 12679436]
107 **Broglio F**, Prodam F, Riganti F, Gottero C, Destefanis S, Granata R, Muccioli G, Abribat T, van der Lely AJ, Ghigo E. The continuous infusion of acylated ghrelin enhances growth hormone secretion and worsens glucose metabolism in humans. *J Endocrinol Invest* 2008; **31**: 788-794 [PMID: 18997491]
108 **Tong J**, Prigeon RL, Davis HW, Bidlingmaier M, Kahn SE, Cummings DE, Tschöp MH, D'Alessio D. Ghrelin suppresses glucose-stimulated insulin secretion and deteriorates glucose tolerance in healthy humans. *Diabetes* 2010; **59**: 2145-2151 [PMID: 20584998 DOI: 10.2337/db10-0504]
109 **Tong J**, Prigeon RL, Davis HW, Bidlingmaier M, Tschöp MH, D'Alessio D. Physiologic concentrations of exogenously infused ghrelin reduces insulin secretion without affecting insulin sensitivity in healthy humans. *J Clin Endocrinol Metab* 2013; **98**: 2536-2543 [PMID: 23589527 DOI: 10.1210/jc.2012-4162]
110 **Alvarez-Castro P**, Isidro ML, García-Buela J, Dieguez C, Casanueva FF, Cordido F. Effect of acute ghrelin administration on glycaemia and insulin levels in obese patients. *Diabetes Obes Metab* 2006; **8**: 555-560 [PMID: 16918590 DOI: 10.1111/j.1463-1326.2006.00551.x]
111 **Benso A**, St-Pierre DH, Prodam F, Gramaglia E, Granata R, van der Lely AJ, Ghigo E, Broglio F. Metabolic effects of overnight continuous infusion of unacylated ghrelin in humans. *Eur J Endocrinol* 2012; **166**: 911-916 [PMID: 22379116 DOI: 10.1530/EJE-11-0982]
112 **Kiewiet RM**, van Aken MO, van der Weerd K, Uitterlinden P, Themmen AP, Hofland LJ, de Rijke YB, Delhanty PJ, Ghigo

E, Abribat T, van der Lely AJ. Effects of acute administration of acylated and unacylated ghrelin on glucose and insulin concentrations in morbidly obese subjects without overt diabetes. *Eur J Endocrinol* 2009; **161**: 567-573 [PMID: 19628651 DOI: 10.1530/EJE-09-0339]

113 **Reimer MK**, Pacini G, Ahrén B. Dose-dependent inhibition by ghrelin of insulin secretion in the mouse. *Endocrinology* 2003; **144**: 916-921 [PMID: 12586768]

114 **Lee HM**, Wang G, Englander EW, Kojima M, Greeley GH. Ghrelin, a new gastrointestinal endocrine peptide that stimulates insulin secretion: enteric distribution, ontogeny, influence of endocrine, and dietary manipulations. *Endocrinology* 2002; **143**: 185-190 [PMID: 11751608]

115 **Salehi A**, Dornonville de la Cour C, Håkanson R, Lundquist I. Effects of ghrelin on insulin and glucagon secretion: a study of isolated pancreatic islets and intact mice. *Regul Pept* 2004; **118**: 143-150 [PMID: 15003830 DOI: 10.1016/j.regpep.2003.12.001]

116 **Qader SS**, Lundquist I, Ekelund M, Håkanson R, Salehi A. Ghrelin activates neuronal constitutive nitric oxide synthase in pancreatic islet cells while inhibiting insulin release and stimulating glucagon release. *Regul Pept* 2005; **128**: 51-56 [PMID: 15721487 DOI: 10.1016/j.regpep.2004.12.018]

117 **de Leeuw van Weenen JE**, Parlevliet ET, Maechler P, Havekes LM, Romijn JA, Ouwens DM, Pijl H, Guigas B. The dopamine receptor D2 agonist bromocriptine inhibits glucose-stimulated insulin secretion by direct activation of the alpha2-adrenergic receptors in beta cells. *Biochem Pharmacol* 2010; **79**: 1827-1836 [PMID: 20138024 DOI: 10.1016/j.bcp.2010.01.029]

118 **Dezaki K**, Sone H, Koizumi M, Nakata M, Kakei M, Nagai H, Hosoda H, Kangawa K, Yada T. Blockade of pancreatic islet-derived ghrelin enhances insulin secretion to prevent high-fat diet-induced glucose intolerance. *Diabetes* 2006; **55**: 3486-3493 [PMID: 17130496 DOI: 10.2337/db06-0878]

119 **Chacko SK**, Haymond MW, Sun Y, Marini JC, Sauer PJ, Ma X, Sunehag AL. Effect of ghrelin on glucose regulation in mice. *Am J Physiol Endocrinol Metab* 2012; **302**: E1055-E1062 [PMID: 22338071 DOI: 10.1152/ajpendo.00445.2011]

120 **Iwakura H**, Hosoda K, Son C, Fujikura J, Tomita T, Noguchi M, Ariyasu H, Takaya K, Masuzaki H, Ogawa Y, Hayashi T, Inoue G, Akamizu T, Hosoda H, Kojima M, Itoh H, Toyokuni S, Kangawa K, Nakao K. Analysis of rat insulin II promoter-ghrelin transgenic mice and rat glucagon promoter-ghrelin transgenic mice. *J Biol Chem* 2005; **280**: 15247-15256 [PMID: 15701644 DOI: 10.1074/jbc.M411358200]

121 **Ma X**, Lin L, Qin G, Lu X, Fiorotto M, Dixit VD, Sun Y. Ablations of ghrelin and ghrelin receptor exhibit differential metabolic phenotypes and thermogenic capacity during aging. *PLoS One* 2011; **6**: e16391 [PMID: 21298106 DOI: 10.1371/journal.pone.0016391]

122 **Sun Y**, Asnicar M, Saha PK, Chan L, Smith RG. Ablation of ghrelin improves the diabetic but not obese phenotype of ob/ob mice. *Cell Metab* 2006; **3**: 379-386 [PMID: 16679295 DOI: 10.1016/j.cmet.2006.04.004]

123 **Longo KA**, Govek EK, Nolan A, McDonagh T, Charoenthongtrakul S, Giuliana DJ, Morgan K, Hixon J, Zhou C, Kelder B, Kopchick JJ, Saunders JO, Navia MA, Curtis R, DiStefano PS, Geddes BJ. Pharmacologic inhibition of ghrelin receptor signaling is insulin sparing and promotes insulin sensitivity. *J Pharmacol Exp Ther* 2011; **339**: 115-124 [PMID: 21775475 DOI: 10.1124/jpet.111.183764]

124 **Ma X**, Lin Y, Lin L, Qin G, Pereira FA, Haymond MW, Butte NF, Sun Y. Ablation of ghrelin receptor in leptin-deficient ob/ob mice has paradoxical effects on glucose homeostasis when compared with ablation of ghrelin in ob/ob mice. *Am J Physiol Endocrinol Metab* 2012; **303**: E422-E431 [PMID: 22669248 DOI: 10.1152/ajpendo.00576.2011]

125 **Doi A**, Shono T, Nishi M, Furuta H, Sasaki H, Nanjo K. IA-2beta, but not IA-2, is induced by ghrelin and inhibits glucose-stimulated insulin secretion. *Proc Natl Acad Sci USA* 2006; **103**: 885-890 [PMID: 16418280 DOI: 10.1073/pnas.0502470102]

126 **Egido EM**, Rodriguez-Gallardo J, Silvestre RA, Marco J. Inhibitory effect of ghrelin on insulin and pancreatic somatostatin secretion. *Eur J Endocrinol* 2002; **146**: 241-244 [PMID: 11834435]

127 **Adeghate E**, Ponery AS. Ghrelin stimulates insulin secretion from the pancreas of normal and diabetic rats. *J Neuroendocrinol* 2002; **14**: 555-560 [PMID: 12121492]

128 **Wierup N**, Yang S, McEvilly RJ, Mulder H, Sundler F. Ghrelin is expressed in a novel endocrine cell type in developing rat islets and inhibits insulin secretion from INS-1 (832/13) cells. *J Histochem Cytochem* 2004; **52**: 301-310 [PMID: 14966197]

129 **Gauna C**, Delhanty PJ, van Aken MO, Janssen JA, Themmen AP, Hofland LJ, Culler M, Broglio F, Ghigo E, van der Lely AJ. Unacylated ghrelin is active on the INS-1E rat insulinoma cell line independently of the growth hormone secretagogue receptor type 1a and the corticotropin releasing factor 2 receptor. *Mol Cell Endocrinol* 2006; **251**: 103-111 [PMID: 16647196 DOI: 10.1016/j.mce.2006.03.040]

130 **Granata R**, Settanni F, Biancone L, Trovato L, Nano R, Bertuzzi F, Destefanis S, Annunziata M, Martinetti M, Catapano F, Ghè C, Isgaard J, Papotti M, Ghigo E, Muccioli G. Acylated and unacylated ghrelin promote proliferation and inhibit apoptosis of pancreatic beta-cells and human islets: involvement of 3′,5′-cyclic adenosine monophosphate/protein kinase A, extracellular signal-regulated kinase 1/2, and phosphatidyl inositol 3-Kinase/Akt signaling. *Endocrinology* 2007; **148**: 512-529 [PMID: 17068144 DOI: 10.1210/en.2006-0266]

131 **Gauna C**, Kiewiet RM, Janssen JA, van de Zande B, Delhanty PJ, Ghigo E, Hofland LJ, Themmen AP, van der Lely AJ. Unacylated ghrelin acts as a potent insulin secretagogue in glucose-stimulated conditions. *Am J Physiol Endocrinol Metab* 2007; **293**: E697-E704 [PMID: 17578884 DOI: 10.1152/ajpendo.00219.2007]

132 **Qader SS**, Håkanson R, Rehfeld JF, Lundquist I, Salehi A. Proghrelin-derived peptides influence the secretion of insulin, glucagon, pancreatic polypeptide and somatostatin: a study on isolated islets from mouse and rat pancreas. *Regul Pept* 2008; **146**: 230-237 [PMID: 17942170 DOI: 10.1016/j.regpep.2007.09.017]

133 **Granata R**, Settanni F, Trovato L, Destefanis S, Gallo D, Martinetti M, Ghigo E, Muccioli G. Unacylated as well as acylated ghrelin promotes cell survival and inhibit apoptosis in HIT-T15 pancreatic beta-cells. *J Endocrinol Invest* 2006; **29**: RC19-RC22 [PMID: 17114904]

134 **Tschöp M**, Wawarta R, Riepl RL, Friedrich S, Bidlingmaier M, Landgraf R, Folwaczny C. Post-prandial decrease of circulating human ghrelin levels. *J Endocrinol Invest* 2001; **24**: RC19-RC21 [PMID: 11434675]

135 **Cummings DE**, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes* 2001; **50**: 1714-1719 [PMID: 11473029]

136 **Caixás A**, Bashore C, Nash W, Pi-Sunyer F, Laferrère B. Insulin, unlike food intake, does not suppress ghrelin in human subjects. *J Clin Endocrinol Metab* 2002; **87**: 1902 [PMID: 11932338]

137 **Broglio F**, Gottero C, Prodam F, Destefanis S, Gauna C, Me E, Riganti F, Vivenza D, Rapa A, Martina V, Arvat E, Bona G, van der Lely AJ, Ghigo E. Ghrelin secretion is inhibited by glucose load and insulin-induced hypoglycaemia but unaffected by glucagon and arginine in humans. *Clin Endocrinol* (Oxf) 2004; **61**: 503-509 [PMID: 15473884 DOI: 10.1111/j.1365-2265.2004.02121.x]

138 **Lucidi P**, Murdolo G, Di Loreto C, De Cicco A, Parlanti N, Fanelli C, Santeusanio F, Bolli GB, De Feo P. Ghrelin is not necessary for adequate hormonal counterregulation of insulin-induced hypoglycemia. *Diabetes* 2002; **51**: 2911-2914

[PMID: 12351426]
139 **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]
140 **Vestergaard ET**, Hansen TK, Nielsen S, Moller N, Christiansen JS, Jorgensen JO. Effects of GH replacement therapy in adults on serum levels of leptin and ghrelin: the role of lipolysis. *Eur J Endocrinol* 2005; **153**: 545-549 [PMID: 16189176 DOI: 10.1530/eje.1.02000]
141 **Flanagan DE**, Evans ML, Monsod TP, Rife F, Heptulla RA, Tamborlane WV, Sherwin RS. The influence of insulin on circulating ghrelin. *Am J Physiol Endocrinol Metab* 2003; **284**: E313-E316 [PMID: 12531744 DOI: 10.1152/ajpendo.00569.2001]
142 **Schaller G**, Schmidt A, Pleiner J, Woloszczuk W, Wolzt M, Luger A. Plasma ghrelin concentrations are not regulated by glucose or insulin: a double-blind, placebo-controlled crossover clamp study. *Diabetes* 2003; **52**: 16-20 [PMID: 12502488]
143 **Rudovich NN**, Dick D, Moehlig M, Otto B, Spranger J, Rochlitz HJ, Ristow M, Tschoep M, Pfeiffer AF. Ghrelin is not suppressed in hyperglycemic clamps by gastric inhibitory polypeptide and arginine. *Regul Pept* 2005; **127**: 95-99 [PMID: 15680475 DOI: 10.1016/j.regpep.2004.10.012]
144 **Paik KH**, Lee MK, Jin DK, Kang HW, Lee KH, Kim AH, Kim C, Lee JE, Oh YJ, Kim S, Han SJ, Kwon EK, Choe YH. Marked suppression of ghrelin concentration by insulin in Prader-willi syndrome. *J Korean Med Sci* 2007; **22**: 177-182 [PMID: 17449920]
145 **Anderwald C**, Brabant G, Bernroider E, Horn R, Brehm A, Waldhäusl W, Roden M. Insulin-dependent modulation of plasma ghrelin and leptin concentrations is less pronounced in type 2 diabetic patients. *Diabetes* 2003; **52**: 1792-1798 [PMID: 12829648]
146 **Micic D**, Sumarac-Dumanovic M, Kendereski A, Cvijovic G, Zoric S, Pejkovic D, Micic J, Milic N, Dieguez C, Casanueva FF. Total ghrelin levels during acute insulin infusion in patients with polycystic ovary syndrome. *J Endocrinol Invest* 2007; **30**: 820-827 [PMID: 18075283]
147 **St-Pierre DH**, Karelis AD, Cianflone K, Conus F, Mignault D, Rabasa-Lhoret R, St-Onge M, Tremblay-Lebeau A, Poehlman ET. Relationship between ghrelin and energy expenditure in healthy young women. *J Clin Endocrinol Metab* 2004; **89**: 5993-5997 [PMID: 15579749 DOI: 10.1210/jc.2004-0613]
148 **Gjedde S**, Vestergaard ET, Gormsen LC, Riis AL, Rungby J, Møller N, Weeke J, Jørgensen JO. Serum ghrelin levels are increased in hypothyroid patients and become normalized by L-thyroxine treatment. *J Clin Endocrinol Metab* 2008; **93**: 2277-2280 [PMID: 18381578 DOI: 10.1210/jc.2007-2619]
149 **Riis AL**, Hansen TK, Møller N, Weeke J, Jørgensen JO. Hyperthyroidism is associated with suppressed circulating ghrelin levels. *J Clin Endocrinol Metab* 2003; **88**: 853-857 [PMID: 12574224]
150 **Kluge M**, Riedl S, Uhr M, Schmidt D, Zhang X, Yassouridis A, Steiger A. Ghrelin affects the hypothalamus-pituitary-thyroid axis in humans by increasing free thyroxine and decreasing TSH in plasma. *Eur J Endocrinol* 2010; **162**: 1059-1065 [PMID: 20423986 DOI: 10.1530/EJE-10-0094]
151 **Gibson W**, Liu J, Gaylinn B, Thorner MO, Meneilly GS, Babich SL, Thompson D, Chanoine JP. Effects of glucose and insulin on acyl ghrelin and desacyl ghrelin, leptin, and adiponectin in pregnant women with diabetes. *Metabolism* 2010; **59**: 841-847 [PMID: 20005544 DOI: 10.1016/j.metabol.2009.09.033]
152 **McCowen KC**, Maykel JA, Bistrian BR, Ling PR. Circulating ghrelin concentrations are lowered by intravenous glucose or hyperinsulinemic euglycemic conditions in rodents. *J Endocrinol* 2002; **175**: R7-11 [PMID: 12429053]

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REVIEW

Clinical therapeutic strategies for early stage of diabetic kidney disease

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Abstract

Diabetic kidney disease (DKD) is the most common cause of chronic kidney disease, leading to end-stage renal disease and cardiovascular disease. The overall number of patients with DKD will continue to increase in parallel with the increasing global pandemic of type 2 diabetes. Based on landmark clinical trials, DKD has become preventable by controlling conventional factors, including hyperglycemia and hypertension, with multifactorial therapy; however, the remaining risk of DKD progression is still high. In this review, we show the importance of targeting remission/regression of microalbuminuria in type 2 diabetic patients, which may protect against the progression of DKD and cardiovascular events. To achieve remission/regression of microalbuminuria, several steps are important, including the early detection of microalbuminuria with continuous screening, targeting HbA1c < 7.0% for glucose control, the use of renin angiotensin system inhibitors to control blood pressure, the use of statins or fibrates to control dyslipidemia, and multifactorial treatment. Reducing microalbuminuria is therefore an important therapeutic goal, and the absence of microalbuminuria could be a pivotal biomarker of therapeutic success in diabetic patients. Other therapies, including vitamin D receptor activation, uric acid-lowering drugs, and incretin-related drugs, may also be promising for the prevention of DKD progression.

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Key words: Diabetic kidney disease; Glycemic control; Renin-angiotensin system inhibitor; Multifactorial therapy; Remission and regression of albuminuria

Core tip: We show the significance of targeting the remission/regression of microalbuminuria in type 2 diabetic patients, leading to protection against the progression of diabetic kidney disease (DKD) and cardiovascular events. To achieve the remission/regression of microalbuminuria, the multifactorial intervention and the early detection of microalbuminuria with continuous screening is important, as management of DKD. Multifactorial intervention includes glucose, blood pressure and lipid control. Additionally, other therapies, including vitamin D receptor activation, uric acid-lowering medicine and incretin-related medicines may be promising for preventing the progression of DKD. We review the current standard treatment for DKD and other prospective therapies for DKD.

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INTRODUCTION

The prevalence of diabetes mellitus is increasing. According to the International Diabetes Federation Atlas of 2012, the estimated diabetes prevalence in 2012 was 371 million, representing 8.3% of the world's adult population; it was predicted that by 2030, the number of people with diabetes in the world will have risen to 552 million[1]. Long-term diabetes results in vascular changes and dysfunction, and diabetic complications are the major causes of morbidity and mortality in diabetic patients. Among diabetic vascular complications, diabetic kidney disease (DKD) is a common cause of chronic kidney disease (CKD) and is a leading cause of end-stage renal disease (ESRD)[2]. In addition, microalbuminuria/proteinuria and a decline in the glomerular filtration rate (GFR) are observed in CKD and are recognized as independent risk factors for the development of ESRD and the onset of cardiovascular diseases, respectively. Therefore, it is important to establish therapeutic strategies for DKD.

The pathogenesis of DKD is complex and has not yet been completely elucidated. Hyperglycemia is one major factor that is responsible for the pathogenesis of DKD[3]. Moreover, elevated systemic blood pressure and intra-glomerular pressure, which are associated with the renin-angiotensin system (RAS), several cytokines and growth factors induced by metabolic and hemodynamic factors, and abnormal lipid metabolism are involved in the pathogenesis of DKD[4,5]. Current therapeutic strategies targeting these mechanisms, particularly the control of blood glucose and blood pressure, have been established in many hallmark clinical trials. In addition, a reduction in microalbuminuria is more frequent than progression to overt proteinuria, and a multifactorial control approach is important for this reduction in microalbuminuria, leading to reductions in renal and cardiovascular risk. In this review, we discuss the current standard treatment and other prospective therapies in DKD (especially early stage) that target a reduction of albuminuria.

MECHANISMS OF ALBUMINURIA IN DKD

Albuminuria is a signature feature of DKD. Albuminuria in DKD is predominantly due to impairment in the glomerular filtration barrier, consisting of the glomerular endothelial cells, the glomerular basement membrane (GBM), and the podocytes[6]. Podocytes are the predominant component of this barrier, and the reduced number of podocytes due to increased apoptosis and detachment from the GBM is observed in the diabetic kidney, resulting in leakage of albumin through areas of denuded podocytes[7-12]. In addition to a decrease in podocyte number and density, the widening of the foot processes, shortening of the slit diaphragm/loss of slit diaphragm proteins, changes in the actin cytoskeleton, and decreases in negative charge may cause albuminuria in DKD[13-15]. Furthermore, endothelial cell injuries in diabetic conditions leading to reduced nitric oxide production[16,17], altered vascular endothelial growth factor (VEGF) signaling[18,19] and diminished glycocalyx[20] also play pivotal roles in albuminuria. Glomerular endothelial cells and podocytes crosstalk through several mediators, including VEGF-A[19], angiopoietin-1[21,22] and -2[23] and activated protein C[24]; therefore, the missing link between endothelial cells and podocytes in diabetic conditions contributes to dysfunction of both cell types, resulting in increased albuminuria[25]. Glomerular hemodynamic changes, including hyperfiltration and hyperperfusion, are observed in diabetic conditions and hypertension. Elevated intraglomerular pressure creates a shear stress on the glomeruli and leads to an increase in albuminuria due to endothelial and podocyte dysfunction[26]. Vascular endothelial dysfunction is closely related to the pathogenesis of the initiation of cardiovascular disease (CVD); albuminuria also reflects glomerular endothelial dysfunction. Therefore, albuminuria is a marker of both glomerular and early systemic endothelial dysfunction[27,28].

Tubular cell injury may also contribute to albuminuria by impairing proximal tubular albumin and protein reabsorption. In diabetes, proximal tubular reuptake of albumin and protein may be impaired by high glucose[29], transforming growth factor (TGF)-β[30], or angiotensin Ⅱ [31]. Tubulointerstitial injury is enhanced and the ability to reabsorb albumin and protein is further reduced, along with the development of glomerular disease, and there is a direct correlation between the degree of tubulointerstitial scarring and the extent of albuminuria[32].

SCREENING METHODS AND DIAGNOSIS OF DIABETIC KIDNEY DISEASE

The early clinical sign of DKD is elevated urinary albumin excretion, referred to as microalbuminuria, which progresses to overt proteinuria and leads to nephritic-range proteinuria in some cases. Increasing albuminuria (proteinuria) leads to a decline in renal function, which is defined in terms of the GFR[33] and generally progresses inexorably to ESRD 6-8 years after the detection of overt proteinuria[34]. Microalbuminuria is defined as a urinary albumin-creatinine ratio (ACR) of 30-299 mg/g creatinine (Cr), and macroalbuminuria is defined as an ACR > 300 mg/g Cr[35]. Elevated ACR should be confirmed in the absence of urinary tract infection in two additional first-void specimens collected during the following 3 to 6 mo[35].

Microalbuminuria in diabetic patients has been recognized as a useful biomarker for diagnosing DKD and as a predictive factor for progression to ESRD. In most patients with diabetes, CKD should be attributed to diabetes if any of the following is true: macroalbuminuria is present, microalbuminuria is present in the presence of diabetic retinopathy, or type 1 diabetes has occurred with a duration of at least 10 years[35]. However, other causes of CKD should be considered in the presence of any of the following circumstances: diabetic retinopathy is absent, GFR is low or rapidly decreasing, proteinuria is increasing or there is evidence of nephritic syndrome,

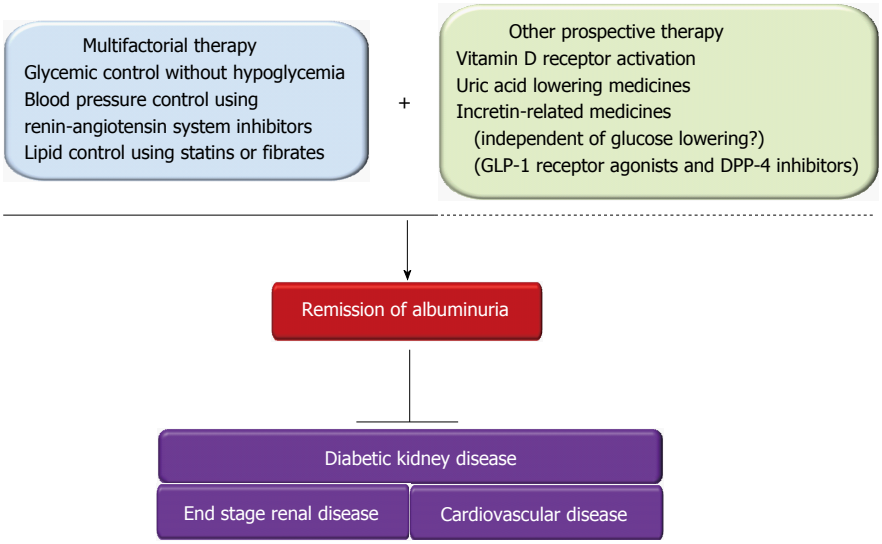

Figure 1 Therapeutic strategy for diabetic kidney disease. Multifactorial therapy, consisting of glycemic, blood pressure, and lipid control, is recommended to prevent the progression of diabetic kidney disease (DKD). The remission and regression of albuminuria by multifactorial therapy may be closely associated with reduced risk of progression of both DKD and cardiovascular disease. In addition to these therapies, vitamin D receptor activation, uric acid-lowering drugs, and incretin-related drugs should be considered in the prospective treatment of DKD.

refractory hypertension is noted, active urinary sediments are present, signs or symptoms of other systemic diseases are present, or a > 30% reduction in GFR has occurred within 2-3 mo after initiation of treatment with an angiotensin converting enzyme (ACE) inhibitor or angiotensin Ⅱ receptor blocker (ARB)[35].

Additionally, microalbuminuria has been shown to be closely associated with an increased risk of cardiovascular morbidity and mortality[36-38]. In a sub-analysis of the United Kingdom Prospective Diabetes Study (UKPDS), the cardiovascular mortality of type 2 diabetic patients with microalbuminuria was reported to be two times higher than that of patients with normoalbuminuria[39]. Therefore, microalbuminuria is not only a biomarker for the diagnosis of DKD but is also an important therapeutic target for improving the prognosis of renal and cardiovascular risk in diabetic patients.

THERAPEUTIC STRATEGY FOR DIABETIC KIDNEY DISEASE

The current therapeutic strategy for DKD is shown in Figure 1. A multifactorial therapeutic approach, including glycemic control, blood pressure management, and lipid control, is recommended to prevent the progression of DKD. The remission and regression of albuminuria as a result of multifactorial therapy may be closely associated with reduced risk of both the progression of DKD and cardiovascular disease. In addition to these therapies, vitamin D receptor activation, uric acid-lowering drugs, and incretin-related drugs are potential treatments for DKD.

BLOOD GLUCOSE CONTROL

Targeting HbA1c

Chronic hyperglycemia is the main causal factor underlying diabetic vascular complications, including DKD. Multiple potential molecular mechanisms have been proposed to explain hyperglycemia-induced diabetic complications. Some of the most-studied mechanisms include disruption of the polyol pathway, activation of the diacylglycerol-protein kinase C pathway, increased oxidative stress, increased formation and activity of advanced glycation end products, and activation of the hexosamine pathway[3]. Additionally, alterations in signal transduction pathways induced by hyperglycemia or toxic metabolites have been reported to cause multiple vascular dysfunctions, such as abnormal blood flow, and increased apoptosis, inflammation, and accumulation of extracellular matrix in the kidney by alteration of gene expression or protein function[3]. Therefore, glycemic control is fundamentally necessary to prevent the onset and progression of DKD by influencing both hyperglycemia itself and hyperglycemia-induced metabolic abnormalities; this premise has been supported by several randomized controlled clinical trials in both type 1 and type 2 diabetes, as described below.

Type 1 diabetes: In the Diabetes Control and Complications Trial (DCCT), the average HbA1c levels were 7% and 9% for the intensive and conventional therapy groups, respectively. Intensive glycemic control was associated with a risk reduction of 34% for the onset of microalbuminuria and a risk reduction of 56% for progression to overt albuminuria[40]. Additionally, in the Epidemiology of Diabetes Interventions and Complications study (the follow-up study to the DCCT), intensive glycemic control prevented the onset of microalbuminuria (yielding a decrease in the odds ratio of 84% for the intensive therapy group) and the progression to overt albuminuria (yielding a decrease in the odds ratio of 59% for the intensive therapy group) at 7-8 years after the end of the DCCT, although the differences in HbA1c

Table 1 Effects of intensive glucose control on the onset and progression of diabetic kidney disease

Study	HbA1c		Outcome of albuminuria or renal events
	Intensive treatment	Conventional treatment	
ACCORD[45]	6.4% *vs* 7.6%		21% ↓ in onset of microalbuminuria 32% ↓ in progression to macroalbuminuria
ADVANCE[46]	6.5% *vs* 7.3%		9% ↓ in onset of microalbuminuria 30% ↓ in progression to macroalbuminuria 21% ↓ in renal events New onset macroalbuminuria Doubling of serum Cr Kidney replacement therapy Death due to kidney disease
VADT[47]	6.9% *vs* 8.4%		32% ↓ in progression from normal to microalbuminuria or macroalbuminuria 37% ↓ in progression from normal to microalbuminuria to macroalbuminuria 34% ↓ in any increase in albuminuria

ACCORD: Action to Control Cardiovascular Risk in Diabetes; ADVANCE: Action in Diabetes and Vascular disease: Preterax and Diamicron MR Controlled Evaluation; VADT: Veterans Affairs Diabetes Trial.

between the intensive and conventional therapy groups had decreased over that time. Moreover, 24 cases exhibited elevated serum Cr levels (≥ 2.0 mg/dL); of these 24 cases, 19 were in the conventional therapy group, and five were in the intensive therapy group[41]. In the follow-up study conducted 22 years after initiation of the DCCT[42], a decrease in the GFR (< 60 mL/min per 1.73 m^2) was observed in the intensive therapy group, with a risk reduction of 50% compared with the conventional therapy group. The decrease in GFR per year was significantly suppressed in the intensive therapy group compared with the conventional therapy group (intensive therapy: conventional therapy, 1.27 mL/min per 1.73 m^2/year: 1.56 mL/min per 1.73 m^2/year).

Type 2 diabetes: In the UKPDS33, the median HbA1c levels were 7.0% and 7.9% for the intensive and conventional therapy groups, respectively. The development of diabetic microvascular complications, including nephropathy, in the intensive therapy group was reduced by 25% relative to the conventional therapy group[43]. In the follow-up study conducted 10 years after the end of the UKPDS, the development of microvascular complications, including nephropathy, in the intensive therapy group was still reduced by 24% compared with the conventional therapy group, although the differences in the HbA1c levels between the intensive and conventional therapy groups had diminished.

In the Kumamoto Study, the average HbA1c levels were 7.5% and 9.8% for the intensive and conventional therapy groups, respectively. The cumulative rates for the development and progression of nephropathy after 6 years were 7.7% for the intensive therapy group and 28.0% for the conventional therapy group in the primary prevention cohort; these rates were 11.5% and 32.0%, respectively, in the secondary intervention cohort. In this study, an HbA1c < 6.9% was identified as the target for preventing the onset and progression of diabetic nephropathy[44]. In the Action to Control Cardiovascular Risk in Diabetes (ACCORD) study, the HbA1c levels at the end of the study were 6.4% and 7.5% for the intensive and conventional therapy groups, respectively. Intensive glycemic control reduced the onset of microalbuminuria by 21% and the progression to macroalbuminuria by 32%[45] (Table 1). In the Action in Diabetes and Vascular disease: Preterax and Diamicron MR Controlled Evaluation (ADVANCE) study, the HbA1c levels at the end of the study were 6.5% and 7.3% for the intensive and conventional therapy groups, respectively. Intensive glycemic control resulted in a 21% reduction in new onset or worsening nephropathy defined by new onset macroalbuminuria, doubling of serum Cr, need for kidney replacement therapy, or death due to kidney disease. Additionally, intensive glycemic control decreased the development of new onset microalbuminuria by 9%, and development of macroalbuminuria by 30%[46] (Table 1). In the Veterans Affairs Diabetes Trial (VADT) study, the HbA1c levels at the end of the study were 6.9% and 8.4% for the intensive and conventional therapy groups, respectively. Intensive glycemic control resulted in a 32% reduction in the progression from normal albuminuria to microalbuminuria or macroalbuminuria, and a 37% reduction in the progression from normal albuminuria to microalbuminuria to macroalbuminuria, and a 34% reduction in any increase in albuminuria[47] (Table 1). The ACCORD, ADVANCE, and VADT studies showed the beneficial effects of intensive glycemic control on the prevention of microalbuminuria and reduced progression to macroalbuminuria; however, these studies showed no significant benefit of more intensive glycemic control on Cr-based estimates of GFR (eGFR).

Based on the results from these clinical trials, the Standards of Medical Care in Diabetes 2014 of the American Diabetes Association (ADA)[33], the Kidney Disease Improving Global Outcomes (KDIGO) 2012 Clinical Practice Guidelines for the Evaluation and Management of Chronic Kidney Disease and the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines for the management of diabetes with CKD[35] recommend a target HbA1c < 7.0%

Table 2 Target of blood pressure in diabetic kidney disease (different of clinical guidelines)

Clinical guideline	Target population	Target of blood pressure
Standard of Medical Care in Diabetes-2014 (ADA)	Diabetic patients	< 140/80 mmHg (< 130 mmHg, younger patients if it can be achieved without undue treatment burden)
KDIGO 2012 CKD guideline	Diabetes + CKD	
	UAE < 30 mg/24 h or ACR < 30 mg/gCr	≤ 140/90 mmHg
	UAE ≥ 30 mg/24 h or ACR ≥ 30 mg/gCr	≤ 130/80 mmHg
JNC8	Diabetic patients	< 140/90 mmHg
	CKD patients	

CKD: Chronic kidney disease; UAE: Urinary albumin excretion; ACR: Albumin creatinine ratio; ADA: American Diabetes Association; KDIGO: The kidney Disease Improving Global Outcomes; JNC8: The Eighth Joint National Committee.

to prevent or delay the progression of DKD. However, clinical evidence that intensive glycemic control reduces DKD is limited to the prevention of microalbuminuria and reduced progression to macroalbuminuria. Evidence of intensive glucose control effecting renal outcomes, including reduced eGFR or the doubling of plasma Cr levels, or on cardiovascular disease, is still ambiguous. Additionally, no reports have prospectively examined the effect of intensive blood glucose control on overt nephropathy with macroalbuminuria, and ESRD or CKD stage 4.

Risk of hypoglycemia

Recent clinical trials, including ADVANCE[46], ACCORD[48], and VADT[47], which reported HbA1c levels of 6.5%, 6.4%, and 6.9%, respectively, showed 1.5-3-fold increases in hypoglycemia in patients with type 2 diabetes who received intensive therapy to reach target glucose levels (with targeted HbA1c levels of < 6.5%, < 6.0%, and < 6.0%, respectively). However, intensive therapy did not decrease the risk of cardiovascular events. Moreover, in the ACCORD study[48], the mortality rates for patients treated with intensive therapy were significantly higher compared to conventional therapy patients. Although the source of the relationship between hypoglycemia and increased mortality in this study was unclear[49], hypoglycemia should be avoided. Therefore, glycemic control without hypoglycemia is important, and the use of glycemic control to target HbA1c levels should be considered in light of the risk factors pertinent to the individual patient, such as the presence of diabetic vascular complications, history of diabetes, and age. At the advanced stage of overt nephropathy with a reduction in renal functioning, the risk of hypoglycemia may be increased because of decreased gluconeogenesis in the kidney, changes in pharmacokinetics resulting from reduced renal function, and reduced insulin metabolism in the kidney. Therefore, it is necessary to select anti-diabetic medicines while considering the individual patient's renal functioning.

BLOOD PRESSURE CONTROL

Targeting blood pressure

Systolic blood pressure control is universally recommended in patients with diabetes to reduce the incidence of stroke, heart failure, diabetes-related death, and retinal photocoagulation, as well as to reduce the risk of the onset of microalbuminuria or progression to overt proteinuria. The early findings from the UKPDS suggest that a 10 mmHg decrease in systolic blood pressure is associated with a reduction of diabetic microvascular complications, including nephropathy, by 13%[50]. Additionally, in the ADVANCE study, a reduction of blood pressure from 140/73 mmHg (control group) to 136/73 mmHg (indapamide-perindopril group) was shown to reduce the risk of a major macro- or microvascular (mostly new microalbuminuria) event and mortality from any cause, including cardiovascular disease[51]. Therefore, the goal of blood pressure < 130/80 mmHg appears to be appropriate in type 2 diabetes to fight against the development and progression of DKD[52]. However, there are recent clinical guidelines for the management of high blood pressure in patients with diabetes and CKD. The KDIGO 2012 Clinical Practice Guidelines for the Evaluation and Management of Chronic Kidney Disease recommends targets for blood pressure in diabetes and CKD as follows. Blood pressure in diabetic adults with CKD and urine albumin excretion < 30 mg/24 h (or ACR < 30 mg/g Cr) should be treated to ≤ 140/90 mmHg, and blood pressure in diabetic adults with CKD and urine albumin excretion ≥ 30 mg/24 h (or ACR ≥ 30 mg/g Cr) should be treated to ≤ 130/80 mmHg. Moreover, the Standards of Medical Care in Diabetes 2014 of the ADA[33] recommends that people with diabetes and hypertension should be treated to < 140/80 mmHg, and lower systolic targets, such as < 130 mmHg, may be appropriate for certain individuals, such as younger patients. However, the 2014 Evidence-Based Guidelines for the Management of High Blood Pressure in Adults from the Panel Members Appointed to the Eighth Joint National Committee (JNC8)[53] recommend a blood pressure goal of < 140/90 mmHg in the population aged ≥ 18 years with CKD or/and diabetes. Thus, recommendations for blood pressure targets differ between the guidelines (Table 2); however, blood control targets should be considered with the risk of the individual patient, such as the presence or absence of other diabetic vascular complications, history of CVD and age,

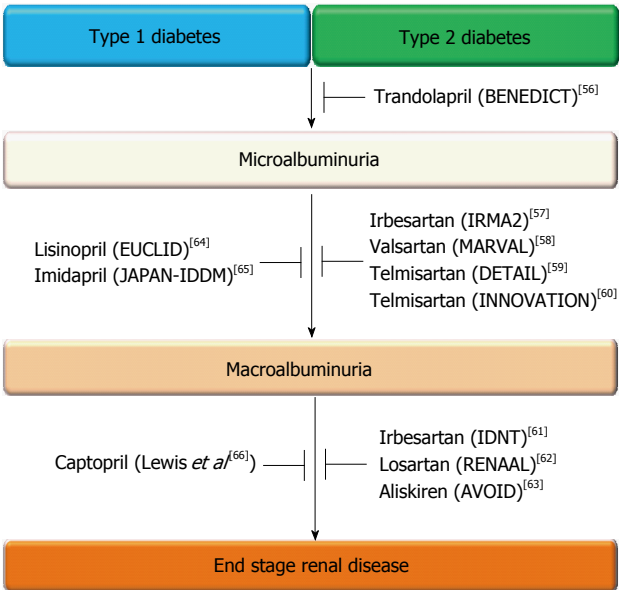

Figure 2 Beneficial effects of renin-angiotensin system inhibitors. Numerous landmark studies have shown the effectiveness of renin-angiotensin system inhibitors on diabetic kidney disease.

as well as glucose control targets.

ACE Inhibitors and ARBs

RAS activation is implicated in the pathogenesis of DKD. In diabetic patients with microalbuminuria or overt proteinuria, RAS inhibitors play a pivotal role in the prevention and treatment of DKD[54,55]. Landmark studies including type 1 and type 2 diabetic patients at various stages of DKD have provided abundant clinical evidence that treatment with RAS inhibitors, including ACE inhibitors and ARBs, slow the progressive decline of GFR, reduce micro- and macroalbuminuria, and reduce cardiovascular mortality and morbidity[54], as shown in Figure 2. Therefore, the use of RAS inhibitors for hypertension and albuminuria in diabetic patients is recommended as a first-line treatment[56-66].

Dual RAS blockade with an ACE inhibitor and ARB may be more effective in reducing proteinuria than monotherapy in patients with DKD. Based on the Ongoing Telmisartan Alone and in Combination with Ramipril Global Endpoint Trial, combination therapy with ramipril and telmisartan reduces proteinuria better than monotherapy; however, it worsens major renal outcomes, including dialysis, the doubling of serum Cr levels, and death[67,68]. Additionally, the Veterans Affairs Nephropathy in Diabetes Clinical Trials showed that combination therapy with an ARB (losartan) and an ACE inhibitor (lisinopril) in type 2 diabetic patients with macroalbuminuria significantly increased the risk of hyperkalemia and acute kidney injury[69]. Thus, combined RAS blockade should not be used in diabetic patients, especially elderly type 2 diabetic patients with normo- or microalbuminuria. First, an ACE inhibitor or ARB should be used, and its dosage should be increased to obtain an optimal anti-albuminuric or proteinuric response. Combination treatment with both an ACE inhibitor and an ARB should be prescribed by a nephrologist and given to patients with overt proteinuria or severe proteinuria, notwithstanding the use of the maximum dosage of the ACE inhibitor or ARBs. In such diabetic patients, monitoring of renal function is necessary, and treatment should be halted in the event of acute kidney injury, low blood pressure, or high potassium levels.

Mineralocorticoid receptor antagonists

Some clinical trials have demonstrated that treatment with spironolactone and eplerenone in addition to an ACE inhibitor or an ARB reduces proteinuria in patients with diabetes[70-75]. However, the long-term effect of mineralocorticoid receptor antagonists on GFR is not clear, and serum potassium levels should be monitored carefully.

Aliskiren

Aliskiren, a direct renin inhibitor, has been promoted for the suppression of DKD and cardiovascular disease. In the Evaluation of Proteinuria in Diabetes study[62], patients with DKD with overt proteinuria were treated with 100 mg of losartan, followed by the addition of a placebo or aliskiren (300 mg). Treatment with 300 mg of aliskiren reduced the mean urinary ACR compared with placebo treatment. However, the Aliskiren Trial in Type 2 Diabetes Using Cardio-Renal Endpoints study[76], which was performed to confirm the effectiveness of combination treatment with either an ACE inhibitor or an ARB plus aliskiren on both renal and cardiovascular events, was terminated because of adverse outcomes, including hyperkalemia and hypotension, and predicted futility in meeting the cardiovascular and renal endpoints.

Calcium channel blockers and diuretics

Because many hypertensive patients with DKD will require a combination therapy to adequately control blood pressure, commonly used combination therapies include an ACE inhibitor or an ARB plus a diuretic or a calcium channel blocker (CCB).

The Gauging Albuminuria Reduction With Lotrel in Diabetic Patients With Hypertension study tested the effect on albuminuria of initial combination therapy of either a dihydropyridine calcium channel blocker or a thiazide diuretic combined with the same ACE inhibitor in patients with type 2 diabetes and hypertension. In the study, both amlodipine and hydrochlorothiazide (HCTZ) combined with an initial treatment using benazepril decreased the median percent change in ACR from baseline to the end of the study; however, the benazepril plus HCTZ group had a greater reduction in albuminuria compared to the benazepril plus amlodipine group (median percent change in ACR: -72.1 *vs* 40.5, $P < 0.0001$)[77]. In contrast, the mean decrease in the eGFR during the observational period was less in the benazepril plus amlodipine group than in the benazepril plus HCTZ group (-2.03 ± 14.2 mL/min *vs* -13.64 ± 16.1 mL/min, $P < 0.0001$)[77].

The Avoiding Cardiovascular Events through Combination Therapy in Patients Living with Systolic Hypertension (ACCOMPLISH) trial was a randomized and double-blind trial in which 11506 patients with hypertension (60% of whom were diabetics) who were at high risk for cardiovascular events were assigned to receive treatment with either benazepril plus amlodipine or benazepril plus HCTZ. The benazepril-amlodipine combination had a relative risk reduction of 19.6% in cardiovascular events[78]. According to the sub-analysis of the ACCOMPLISH trial on renal outcomes, the events of CKD progression defined as a doubling of serum Cr concentration or ESRD (eGFR < 15 mL/min per 1.73 m^2 or need for dialysis) occurred at a frequency of 2.0% in the benazepril plus amlodipine group compared to 3.7% in the benazepril plus HCTZ group (HR = 0.52, 0.41-0.65, $P < 0.0001$). However, in the patients with CKD (more than half of patients have DKD), both the progression of CKD and cardiovascular mortality did not differ between groups[79].

It is still unclear which additional anti-hypertensive drug (CCB or diuretic) is better for providing both reno- and cardioprotection in DKD. Therefore, the risk of the individual patient, such as the history of CVD and age, should be taken into consideration.

LIPID CONTROL

Dyslipidemia, statins, and fibrates

Dyslipidemia is a major risk factor for atherosclerotic cardiovascular disease, which is a cause of mortality and morbidity in patients with diabetes and CKD[80,81]. In particular, low-density lipoprotein cholesterol (LDL-C) plays an important role in the development of coronary artery disease. Several clinical trials using statin-based lipid-lowering therapies in patients with CKD and diabetes have shown reductions in the risk of major atherosclerotic events. In addition to reducing the risk of cardiovascular diseases in CKD patients, evidence suggests that statin therapy in patients with predialysis CKD may slow the progressive loss of kidney function, measured as changes in urinary albumin/protein excretion or eGFR[82-89]. In the Collaborative Atorvastatin in Diabetes Study, atorvastatin (10 mg/d) treatment was associated with increased GFR in comparison with a placebo, and a modest beneficial effect was observed, particularly in patients with albuminuria. Moreover, atorvastatin was effective at decreasing cardiovascular disease (by 42%) in patients with a moderately decreased eGFR (30-60 mL/min per 1.73 m^2), and this treatment effect was similar to the 37% reduction in cardiovascular disease observed in patients without decreased eGFR[90]. Furthermore, a meta-analysis showed that statin therapy was associated with decreased albuminuria compared to a placebo[87].

The Fenofibrate Intervention and Event Lowering in Diabetes study demonstrated that fenofibrate (200 mg/d) reduced cardiovascular events, reduced albuminuria, and slowed eGFR loss over 5 years, although it initially and reversibly increased plasma Cr levels. In a meta-analysis, fibrates reduced the risk of albuminuria progression in patients with diabetes and reduced the risk of major cardiovascular events and cardiovascular death in patients with an eGFR of 30-59.9 mL/min per 1.73 m^2[91,92].

Statins and fibrates can exert renoprotective effects pleiotropically, such as anti-oxidant, anti-inflammation, and anti-fibrotic effects, independent of their lipid-lowering effects, in experimental animal models[93,94].

KDOQI guidelines and the ADA recommend that the LDL-C target in patients with diabetes or/and CKD should be < 100 mg/dL, and a lower LDL-C goal of < 70 mg/dL is a therapeutic option in individuals with overt CVD, by treatment with statins. Triglyceride levels < 150 mg/dL and high-density lipoprotein cholesterol (HDL-C) > 40 mg/dL in males and > 50 mg/dL in females are desirable[33,35].

MULTIFACTORIAL INTENSIVE THERAPY

Effects on the progression of diabetic kidney disease

The Steno-2 study showed the effect of multifactorial intensive therapy on the progression of nephropathy in patients with type 2 diabetes[95]. In this study, 160 patients with type 2 diabetes and microalbuminuria (average age, 55 years) were randomly divided, with 80 patients assigned to a standard therapy group and 80 patients assigned to an intensive therapy group. The progression of nephropathy was evaluated as a secondary end point. During the 1993-1999 period, the targets for glycemic control, systolic blood pressure, diastolic blood pressure, total cholesterol levels, and triglyceride levels were < 6.5%, < 140 mmHg, < 85 mmHg, < 190 mg/dL, and < 150 mg/dL, respectively, in the intensive therapy group. Patients were administered ARB or ACE inhibitors (regardless of their blood pressure); patients with ischemic heart disease or peripheral vascular disease were given aspirin, and supplementation with vitamin C and E was also provided. Additionally, diet therapy (lipid restriction, < 30% of energy intake per day and < 10% from saturated fatty acid intake) and exercise therapy (3-5 times/wk, moderately intense activity) were prescribed. In the 2000-2001 period, the targets for fasting total cholesterol levels, systolic blood pressure, and diastolic blood pressure were changed to < 175 mg/dL, < 130 mmHg, and < 80 mmHg, respectively, because the treatment guidelines in Denmark changed. In the average observation period of 7.8 years, HbA1c; systolic and diastolic blood pressure; total cholesterol, LDL-C, and triglyceride levels; and fat intake were significantly reduced in the intensive therapy group compared with the standard therapy group. Moreover, the use of aspirin was significantly higher in the intensive therapy group, and urinary albumin excretion was significantly decreased in the intensive therapy group (46 mg/d) compared with the standard therapy group (126 mg/d). Moreover, the risk of onset and progression of nephropathy was reduced to a hazard ratio of 0.39 (CI: 0.17-0.87).

Furthermore, after the Steno-2 study, 63 patients in the standard therapy group underwent intensive therapy with 67 patients of the intensive therapy group in the average follow-up period of 5.5 years[96]. In the follow-up study, the onset and progression of nephropathy were assessed as secondary endpoints. At the end of the follow-up period, glucose, blood pressure, and lipid control in the standard therapy group were improved to almost the same levels as in the intensive therapy group. However, for the total observation period of 13.3 years combined with an average follow-up period of 7.8 years, the onset and progression of nephropathy were decreased in the intensive therapy group [HR = 0.44 (CI: 0.25-0.77)]. Six cases and one case progressed to ESRD in the standard and intensive therapy groups, respectively ($P = 0.04$).

Additionally, a cohort study with a 4-year follow-up of 1290 type 2 diabetic patients with normal albuminuria was performed using multifactorial intensive therapy[97]. In this cohort study, the targets of blood glucose, blood pressure, LDL and triglyceride levels were as follows: HbA1c < 7.0%, < 130/80 mmHg, < 100 mg/dL, < 150 mg/dL, and HDL ⩾ 40 mg/dL (male) per 50 mg/mg per deciliter (female). New microalbuminuria appeared in 211 patients (16.4%) and HbA1c levels < 7% (HR = 0.729, 95%CI: 0.553-0.906, $P = 0.03$), blood pressure < 130 mmHg [HR = 0.645 (CI: 0.491-0.848), HDL ⩾ 40 mg/dL (male) per 50 mg/dL (female), HR = 0.715 (CI: 0.537-0.951)] were associated with the onset of albuminuria.

Accordingly, multifactorial intensive therapy is recommended for suppressing the onset and progression of early diabetic nephropathy; however, it should be noted that this recommendation is based on a small RCT. Moreover, the suppressive effect of multifactorial intensive therapy on nephropathy is not clear in the advanced stage of overt nephropathy.

Effects on the onset of cardiovascular events

In the Steno-2 study described above, the incidence of cardiovascular diseases, including cardiovascular death, non-fatal myocardial infarction, non-fatal stroke, revascularization, and amputation, were evaluated as the primary endpoints over 7.8 years[95]. Thirty-three cardiovascular events (24%) in 19 cases were observed for the intensive therapy group; conversely, 35 cardiovascular events (40%) were observed in the standard therapy group. These results indicate that the risk of cardiovascular disease in type 2 diabetic patients with microalbuminuria was significantly reduced after multifactorial intensive therapy compared with standard therapy [HR = 0.47 (CI: 0.24-0.73)].

In the Steno-2 follow-up study, performed for an average of 5.5 years in addition to the original 7.8 years, the incidence of lower limb amputation, nonfatal stroke, nonfatal myocardial infarction, coronary artery bypass grafting, and percutaneous transluminal coronary angioplasty were assessed as the primary endpoints[96]. At the end of the follow-up period, glycemia, blood pressure, and lipid control for the standard therapy group had improved to levels similar to those found in the intensive therapy group. However, for the total observation period of 13.3 years, the onset of cardiovascular disease was decreased in the intensive therapy group. In addition, there were 48 cases and 158 cardiovascular events in the standard therapy group, in contrast to 28 cases and 51 cardiovascular events in the intensive therapy group.

Remission and regression of albuminuria

Reduction of microalbuminuria in diabetic patients occurred more frequently than we expected. Araki *et al*[98] reported that microalbuminuria in type 2 diabetic patients could improve to normoalbuminuria (remission) or could decrease by more than 50% from the baseline (regression) based on the results of a prospective observational follow-up study over a 6-year period. The 6-year cumulative incidence of progression from microalbuminuria to overt proteinuria was 28% (95%CI: 19%-37%), whereas the remission and regression rates were 51% (95%CI: 42%-60%) and 54% (95%CI: 45%-63%), respectively (Figure 2). In a pooled logistic regression analysis, each modifiable factor was trisected according to the number of patients and was applied as three categories in the analysis. The results showed that microalbuminuria of short duration, the use of RAS blockade, HbA1c < 7.35%, and lower systolic blood pressure (< 130 mmHg) were identified as independent factors associated with remission/regression of microalbuminuria.

ARBs have also been shown to induce remission and regression of microalbuminuria in type 2 diabetic patients. In the Incipient to Overt: Angiotensin Ⅱ Blocker, Telmisartan, Investigation on Type 2 Diabetic Nephropathy study, remission of microalbuminuria at the final observation point occurred in 21.2% of patients treated with 80 mg of telmisartan, 12.8% of patients treated with 40 mg of telmisartan, and 1.2% of patients given a placebo (both telmisartan doses *vs* placebo, $P < 0.001$)[58]. Additionally, patients receiving 80 or 40 mg of telmisartan achieved superior renoprotection, as indicated by lower transition rates to overt nephropathy compared to the placebo patients. Taken together, these results strongly indicate that RAS blockade using an ARB not only prevents the progression of microalbuminuria to overt proteinuria but also induces remission and regression of microalbuminuria in type 2 diabetic patients.

The Steno-2 study also demonstrated that a high proportion of patients with microalbuminuria returned to normoalbuminuria through the multifactorial intervention. After a mean of 7.8 years of follow-up, 46 (31%) patients returned to normoalbuminuria, 58 (38%) patients still had microalbuminuria, and 47 (31%) patients progressed to overt proteinuria[99]. Lower HbA1c levels, initiation of antihypertensive therapy, and initiation of RAS inhibitors during the follow-up period were independently associated with remission of microalbuminuria. A recent analysis focusing particularly on the effect of lowering blood pressure clearly showed that more than half of all type 2 diabetic patients with microalbuminuria and macroalbuminuria returned to normoalbuminuria with receiving any blood pressure-lowering drugs in the

ADVANCE study[100]. However, more patients achieved remission to normoalbuminuria in the perindopril-indapamide treatment group than in the placebo treatment group.

Clinical impact of the remission and regression of albuminuria on cardiovascular events and kidney function

The clinical impact of the remission and regression of microalbuminuria was demonstrated by the observed reduction in the risk of renal and cardiovascular events during an expanded 2-year follow-up (beyond the initial 6 years of the study reported by Araki *et al*[101], described above). The primary outcome measure consisted of "combined incidence," defined as cardiovascular death by and first hospitalization for renal and cardiovascular events. A secondary outcome was kidney function, as determined by the annual decline of eGFR. During the total 8-year follow-up period, 47 patients experienced primary renal and cardiovascular events. Eleven first occurrences of outcomes occurred in subgroups that achieved remission of microalbuminuria; in contrast, 36 such events were observed for the non-remission group. The pooled logistic analysis, adjusted for sex, age, initial ACR levels, history of cardiovascular disease, current smoking, HbA1c level, total cholesterol level, blood pressure, use of RAS inhibitors, use of lipid-lowering drugs, and body mass index, showed that the relative risk for outcomes in patients who achieved remission was 0.25 (95%CI: 0.07-0.87) compared with those whose microalbuminuric status did not change during the follow-up period, whereas the relative risk for patients who progressed to overt proteinuria was 2.55 (95%CI: 1.04-6.30) (Figure 2). First occurrences of these outcomes were classified into subgroups defined by achieving a reduction greater than 50% in urinary albumin excretion in the course of 12 events for the regression group and in 35 events in the non-regression group; these patients were labeled as having failed to achieve remission.

Kaplan-Meier estimations showed that the cumulative incidence of evaluated events was significantly lower in the regression group than in the non-regression group. The 8-year cumulative incidence of these outcomes in the regression group showed a 59% decrease compared to the non-regression group. The adjusted risk for outcomes in patients who achieved regression was 0.41 (95%CI: 0.15-0.96) compared with those whose microalbuminuric status did not show regression during the follow-up. As anticipated, the annual decline of eGFR for the progression group (median: 4.2 mL/min per year) was significantly faster than that for the non-change group (2.4 mL/min per year), whereas the annual decline of eGFR for the remission group was significantly slower (1.1 mL/min per year) and was almost identical to the decline experienced through normal aging reported in healthy people[102].

The effect of reducing microalbuminuria on kidney functioning was also shown in a secondary analysis of the Steno-2 study[101]. The patients who reverted to normoalbuminuria had an average eGFR decrease of 2.3 mL/min per year; however, those who still had microalbuminuria experienced an average eGFR decrease of 3.7 mL/min per year, and those who progressed to overt proteinuria showed the highest eGFR decline of 5.4 mL/min per year. These results show that remission of microalbuminuria is closely related to the improved renal functioning over the long term.

OTHER PROSPECTIVE THERAPEUTIC STRATEGIES

Vitamin D receptor activation

Stimulation of vitamin D receptors exerts protective activity through multiple mechanisms, including inhibition of the RAS, regulation of proliferation and differentiation, reduction of proteinuria, anti-inflammation, and anti-fibrosis[103]. Growing evidence indicates that vitamin D exerts anti-proteinuric and renoprotective effects in DKD patients. The VITAL study demonstrated that treatment with paricalcitol, a selective vitamin D receptor activator, reduced urinary albumin excretion in type 2 diabetic patients treated with RAS inhibitors[104]. Additionally, Kim *et al*[105] showed beneficial effects of vitamin D (cholecalciferol) repletion on urinary albumin and transforming growth factor-β_1 excretion in type 2 diabetic patients with CKD undergoing established RAS inhibition therapy; similar effects were also observed in the VITAL study. Treatment with cholecalciferol led to significantly higher levels of circulating 25(OH)D and $1,25(OH)_2D_3$ relative to baseline, and increased levels of active forms of vitamin D were correlated with a decrease in urinary ACR and TGF-β_1 at the end of a 4-mo intervention period. These data indicate that vitamin D compounds may be useful tools for delaying the progression of DKD beyond the effects expected from established RAS inhibition protocols.

Uric acid-lowering drugs

Multiple longitudinal cohort studies have shown that elevated serum uric acid levels are associated with a higher risk of the onset and progression of microalbuminuria in addition to sustained decline of GFR among type 1 diabetic patients[106-108]. In a cohort study of 263 newly diagnosed type 1 diabetic patients performed by the Steno Diabetes Center group[106], serum uric acid levels measured shortly after the onset of type 1 diabetes were a significant independent predictor of macroalbuminuria 18 years later (HR = 2.37, 95%CI: 1.04-5.37, P = 0.04). Additionally, the Coronary Artery Calcification in Type 1 Diabetes study showed that serum uric acid levels predicted the transition from microalbuminuria to macroalbuminuria[107]. In 324 type 1 diabetic patients, every 1 mg/dL increase in uric acid levels at baseline was associated with an 80% increase in the predicted odds ratio of developing microalbuminuria or macroalbuminuria after 6 years of follow-up (OR = 1.8, 95%CI: 1.2-2.8, P = 0.005). A 6-year follow-up of a prospective cohort study

of type 1 diabetic patients without proteinuria conducted by the Joslin Diabetes Center demonstrated a significant association ($P < 0.0002$) between serum uric acid and an early decrease in GFR, defined as a GFR cystatin decrease exceeding 3.3% per year[108]. When baseline uric acid concentrations were treated categorically (in mg/dL: < 3.0, 3.0-3.9, 4.0-4.9, 5.0-5.9, and ≥ 6), the risk of early decrease in GFR increased linearly (9%, 13%, 20%, 29%, and 36%, respectively). This linear increase corresponds to an OR of 1.4 (95%CI: 1.1-1.8) per 1 mg/dL increase in uric acid levels.

Furthermore, a post-hoc analysis of the Reduction of Endpoints in non-Insulin Dependent Diabetes Mellitus with the Angiotensin Ⅱ Antagonist Losartan trial showed that the decrease in serum uric acid levels induced by losartan accounted for 20% of the renoprotective benefit provided by this medication[109]. However, it is not clear whether reducing uric acid levels could prevent or delay GFR decline in diabetic patients who are at high risk for the progression of DKD; therefore, clinical trials are necessary to elucidate the beneficial effects of uric acid-lowering medicine on preventing DKD.

GLP-1 receptor agonists and DPP-4 inhibitors

Incretin-related therapies, including dipeptidyl peptidase (DPP)-4 inhibitors and glucagon-like peptide (GLP)-1 receptor agonists, have been developed as one of the most promising treatments for type 2 diabetes because of their effectiveness at reducing glucose levels with a low risk of hypoglycemia and no weight gain[110-112]. DPP-4 inhibitors increase the concentration of endogenous incretins, such as GLP-1 and glucose-dependent insulinotropic polypeptides, and GLP-1 analogues that are not degraded by DPP-4 may stimulate GLP-1 receptors in turn. Stimulation of GLP-1 receptors increases glucose-dependent insulin secretion from pancreatic β-cells and suppresses glucagon release from α-cells, leading to improved glucose control[110]. In addition to its action on the pancreas, GLP-1 may have direct effects on other cells and tissues, including the kidney, heart, and blood vessels, *via* stimulation of the GLP-1 receptor[113,114], independent of its glucose-lowering effects.

The GLP-1 receptors in the kidney are expressed in the glomerular endothelial cells, mesangial cells, and proximal tubular cells[115-120], and previous reports have shown that the expression of GLP-1 receptors decreases in the diabetic kidneys of animal models[115]. The renoprotective effect of GLP-1 may be accomplished through anti-inflammation[116], anti-oxidants mediated through cyclic AMP-mediated protein kinase A activation[117,120], or blood pressure regulation *via* sodium handling in proximal tubular cells[121]. DPP-4 is expressed in renal tubular cells, especially in the brush-border and microvillus fractions, podocytes, and endothelial cells[122,123]; however, the physiological role of DPP-4 in the kidney has not been elucidated. Previous reports have shown that DPP-4 expression is increased in the diabetic kidneys of animal models[124]. DPP-4 is a serine exopeptidase that cleaves X-proline dipeptides from the N-terminus of polypeptides. Therefore, DPP-4 cleaves not only incretins but also many substrates, such as cytokines, chemokines, hormones, and neuropeptides[125]. Among these substrates, high-mobility group protein-B1, meprin β, and neuropeptide Y have been identified as candidate targets for GLP-1-independent effects of DPP-4 inhibitors in the kidneys[114].

Several clinical studies have shown beneficial effects of DPP-4 inhibitors[126,127] and GLP-1 analogues[128] on albuminuria in type 2 diabetic patients. Recent reports have demonstrated that linagliptin administration in addition to stable RAS inhibition leads to a significant reduction in type 2 diabetes with albuminuria and renal dysfunction, independent of changes in glucose levels or systolic blood pressure[129]. Further studies, including randomized controlled clinical trials in large populations, are necessary to confirm the long-term effects of incretin-related medicines in DKD.

CONCLUSION

Reduced microalbuminuria may be frequent in diabetic patients. Physicians have to care for these diabetic patients with an aggressive multifactorial management plan as early as possible after the development of microalbuminuria. This multifactorial management regimen includes glycemic control without triggering hypoglycemia, blood pressure control using RAS inhibitors, and lipid control using statins or fibrates. In addition to these therapies, vitamin D receptor activators, uric acid-lowering drugs, and incretin-related drugs for glycemic control are promising therapies for stopping the progression of DKD. However, in the future, the development of novel therapies that not only function to prevent renal decline but also simultaneously attenuate CVD are necessary because the current multifactorial treatment is not still enough.

The remission or regression of microalbuminuria results in reduced risk of both renal and cardiovascular events; therefore, albuminuria is a useful biomarker for the diagnosis of DKD and the assessment of therapeutic effects for DKD. However, some patients with diabetes have advanced renal pathological changes and progressive kidney function decline even though urinary albumin levels are in the normal range, indicating that albuminuria is not the perfect biomarker for early detection of DKD[130]. Recent studies have provided some possible new markers for DKD in type 1[131,132] and type 2 diabetic patients[133]. Serum concentrations of the soluble receptors 1 and 2 for Tissue Necrosis Factor (sTNFR1 and sTNFR2) had a stronger correlation with decline in GFR than urinary ACR[131,132]. sTNFR1 was associated with the development of ESRD in type 2 patients during a 12 year follow-up[133]. However, additional clinical data about such new biomarkers for the early diagnosis and prediction of DKD should be accumulated, and at the same time, it is necessary to determine whether the new biomarker is a

predictive marker for CVD.

REFERENCES

1 **International Diabetes Federation**. IDF Diabetes Atlas. 2012. Available from: URL: http: //www.idf.org/diabetesatlas/5e/Update2012

2 **Packham DK**, Alves TP, Dwyer JP, Atkins R, de Zeeuw D, Cooper M, Shahinfar S, Lewis JB, Lambers Heerspink HJ. Relative incidence of ESRD versus cardiovascular mortality in proteinuric type 2 diabetes and nephropathy: results from the DIAMETRIC (Diabetes Mellitus Treatment for Renal Insufficiency Consortium) database. *Am J Kidney Dis* 2012; **59**: 75-83 [PMID: 22051245 DOI: 10.1053/j.ajkd.2011.09.017]

3 **Kitada M**, Zhang Z, Mima A, and King GL. Molecular mechanisms of diabetic vascular complications. *J Diabetes Invest* 2010; **1**: 77-89 [DOI: 10.1111/j.2040-1124.2010.00018.x]

4 **Forbes JM**, Cooper ME. Mechanisms of diabetic complications. *Physiol Rev* 2013; **93**: 137-188 [PMID: 23303908 DOI: 10.1152/physrev.00045.2011]

5 **Giunti S**, Barit D, Cooper ME. Mechanisms of diabetic nephropathy: role of hypertension. *Hypertension* 2006; **48**: 519-526 [PMID: 16952978 DOI: 10.1161/01.HYP.0000240331.32352.0c]

6 **Jefferson JA**, Shankland SJ, Pichler RH. Proteinuria in diabetic kidney disease: a mechanistic viewpoint. *Kidney Int* 2008; **74**: 22-36 [PMID: 18418356 DOI: 10.1038/ki.2008.128]

7 **Meyer TW**, Bennett PH, Nelson RG. Podocyte number predicts long-term urinary albumin excretion in Pima Indians with Type II diabetes and microalbuminuria. *Diabetologia* 1999; **42**: 1341-1344 [PMID: 10550418 DOI: 10.1007/s001250051447]

8 **Pagtalunan ME**, Miller PL, Jumping-Eagle S, Nelson RG, Myers BD, Rennke HG, Coplon NS, Sun L, Meyer TW. Podocyte loss and progressive glomerular injury in type II diabetes. *J Clin Invest* 1997; **99**: 342-348 [PMID: 9006003 DOI: 10.1172/JCI119163]

9 **White KE**, Bilous RW, Marshall SM, El Nahas M, Remuzzi G, Piras G, De Cosmo S, Viberti G. Podocyte number in normotensive type 1 diabetic patients with albuminuria. *Diabetes* 2002; **51**: 3083-3089 [PMID: 12351451 DOI: 10.2337/diabetes.51.10.3083]

10 **Dalla Vestra M**, Masiero A, Roiter AM, Saller A, Crepaldi G, Fioretto P. Is podocyte injury relevant in diabetic nephropathy? Studies in patients with type 2 diabetes. *Diabetes* 2003; **52**: 1031-1035 [PMID: 12663476 DOI: 10.2337/diabetes.52.4.1031]

11 **Petermann AT**, Pippin J, Krofft R, Blonski M, Griffin S, Durvasula R, Shankland SJ. Viable podocytes detach in experimental diabetic nephropathy: potential mechanism underlying glomerulosclerosis. *Nephron Exp Nephrol* 2004; **98**: e114-e123 [PMID: 15627794 DOI: 10.1159/000081555]

12 **Susztak K**, Raff AC, Schiffer M, Böttinger EP. Glucose-induced reactive oxygen species cause apoptosis of podocytes and podocyte depletion at the onset of diabetic nephropathy. *Diabetes* 2006; **55**: 225-233 [PMID: 16380497 DOI: 10.2337/diabetes.55.01.06.db05-0894]

13 **Doublier S**, Salvidio G, Lupia E, Ruotsalainen V, Verzola D, Deferrari G, Camussi G. Nephrin expression is reduced in human diabetic nephropathy: evidence for a distinct role for glycated albumin and angiotensin II. *Diabetes* 2003; **52**: 1023-1030 [PMID: 12663475 DOI: 10.2337/diabetes.52.4.1023]

14 **Langham RG**, Kelly DJ, Cox AJ, Thomson NM, Holthöfer H, Zaoui P, Pinel N, Cordonnier DJ, Gilbert RE. Proteinuria and the expression of the podocyte slit diaphragm protein, nephrin, in diabetic nephropathy: effects of angiotensin converting enzyme inhibition. *Diabetologia* 2002; **45**: 1572-1576 [PMID: 12436341 DOI: 10.1007/s00125-002-0946-y]

15 **Mifsud SA**, Allen TJ, Bertram JF, Hulthen UL, Kelly DJ, Cooper ME, Wilkinson-Berka JL, Gilbert RE. Podocyte foot process broadening in experimental diabetic nephropathy: amelioration with renin-angiotensin blockade. *Diabetologia* 2001; **44**: 878-882 [PMID: 11508273 DOI: 10.1007/s001250100561]

16 **Nakagawa T**, Sato W, Glushakova O, Heinig M, Clarke T, Campbell-Thompson M, Yuzawa Y, Atkinson MA, Johnson RJ, Croker B. Diabetic endothelial nitric oxide synthase knockout mice develop advanced diabetic nephropathy. *J Am Soc Nephrol* 2007; **18**: 539-550 [PMID: 17202420 DOI: 10.1681/ASN.2006050459]

17 **Rask-Madsen C**, King GL. Vascular complications of diabetes: mechanisms of injury and protective factors. *Cell Metab* 2013; **17**: 20-33 [PMID: 23312281 DOI: 10.1016/j.cmet.2012.11.012]

18 **Hohenstein B**, Hausknecht B, Boehmer K, Riess R, Brekken RA, Hugo CP. Local VEGF activity but not VEGF expression is tightly regulated during diabetic nephropathy in man. *Kidney Int* 2006; **69**: 1654-1661 [PMID: 16541023 DOI: 10.1038/sj.ki.5000294]

19 **Singh A**, Satchell SC, Neal CR, McKenzie EA, Tooke JE, Mathieson PW. Glomerular endothelial glycocalyx constitutes a barrier to protein permeability. *J Am Soc Nephrol* 2007; **18**: 2885-2893 [PMID: 17942961 DOI: 10.1681/ASN.2007010119]

20 **Advani A**. Vascular endothelial growth factor and the kidney: something of the marvellous. *Curr Opin Nephrol Hypertens* 2014; **23**: 87-92 [PMID: 24247821 DOI: 10.1097/01.mnh.0000437329.41546.a9]

21 **Jeansson M**, Gawlik A, Anderson G, Li C, Kerjaschki D, Henkelman M, Quaggin SE. Angiopoietin-1 is essential in mouse vasculature during development and in response to injury. *J Clin Invest* 2011; **121**: 2278-2289 [PMID: 21606590 DOI: 10.1172/JCI46322]

22 **Dessapt-Baradez C**, Woolf AS, White KE, Pan J, Huang JL, Hayward AA, Price KL, Kolatsi-Joannou M, Locatelli M, Diennet M, Webster Z, Smillie SJ, Nair V, Kretzler M, Cohen CD, Long DA, Gnudi L. Targeted glomerular angiopoietin-1 therapy for early diabetic kidney disease. *J Am Soc Nephrol* 2014; **25**: 33-42 [PMID: 24009238 DOI: 10.1681/ASN.2012121218]

23 **Davis B**, Dei Cas A, Long DA, White KE, Hayward A, Ku CH, Woolf AS, Bilous R, Viberti G, Gnudi L. Podocyte-specific expression of angiopoietin-2 causes proteinuria and apoptosis of glomerular endothelia. *J Am Soc Nephrol* 2007; **18**: 2320-2329 [PMID: 17625119 DOI: 10.1681/ASN.2006101093]

24 **Isermann B**, Vinnikov IA, Madhusudhan T, Herzog S, Kashif M, Blautzik J, Corat MA, Zeier M, Blessing E, Oh J, Gerlitz B, Berg DT, Grinnell BW, Chavakis T, Esmon CT, Weiler H, Bierhaus A, Nawroth PP. Activated protein C protects against diabetic nephropathy by inhibiting endothelial and podocyte apoptosis. *Nat Med* 2007; **13**: 1349-1358 [PMID: 17982464 DOI: 10.1038/nm1667]

25 **Siddiqi FS**, Advani A. Endothelial-podocyte crosstalk: the missing link between endothelial dysfunction and albuminuria in diabetes. *Diabetes* 2013; **62**: 3647-3655 [PMID: 24158990 DOI: 10.2337/db13-0795]

26 **Wolf G**. New insights into the pathophysiology of diabetic nephropathy: from haemodynamics to molecular pathology. *Eur J Clin Invest* 2004; **34**: 785-796 [PMID: 15606719 DOI: 10.1111/j.1365-2362.2004.01429.x]

27 **Deckert T**, Kofoed-Enevoldsen A, Nørgaard K, Borch-Johnsen K, Feldt-Rasmussen B, Jensen T. Microalbuminuria. Implications for micro- and macrovascular disease. *Diabetes Care* 1992; **15**: 1181-1191 [PMID: 1396015 DOI: 10.2337/diacare.15.9.1181]

28 **Satoh M**. Endothelial dysfunction as an underlying pathophysiological condition of chronic kidney disease. *Clin Exp Nephrol* 2012; **16**: 518-521 [PMID: 22669535 DOI: 10.1007/s10157-012-0646-y]

29 **Ishibashi F**. High glucose reduces albumin uptake in cultured proximal tubular cells (LLC-PK1). *Diabetes Res Clin Pract* 2004; **65**: 217-225 [PMID: 15331201 DOI: 10.1016/j.diabres.2004.02.003]

30 **Russo LM**, del Re E, Brown D, Lin HY. Evidence for a role of transforming growth factor (TGF)-beta1 in the induction of

postglomerular albuminuria in diabetic nephropathy: amelioration by soluble TGF-beta type II receptor. *Diabetes* 2007; **56**: 380-388 [PMID: 17259382 DOI: 10.2337/db06-1018]
31 **Clavant SP**, Forbes JM, Thallas V, Osicka TM, Jerums G, Comper WD. Reversible angiotensin II-mediated albuminuria in rat kidneys is dynamically associated with cytoskeletal organization. *Nephron Physiol* 2003; **93**: p51-p60 [PMID: 12629271 DOI: 10.1159/000068528]
32 **Gilbert RE**, Cooper ME. The tubulointerstitium in progressive diabetic kidney disease: more than an aftermath of glomerular injury? *Kidney Int* 1999; **56**: 1627-1637 [PMID: 10571771 DOI: 10.1046/j.1523-1755.1999.00721.x]
33 **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]
34 **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]
35 **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]
36 **Garg JP**, Bakris GL. Microalbuminuria: marker of vascular dysfunction, risk factor for cardiovascular disease. *Vasc Med* 2002; **7**: 35-43 [PMID: 12083733 DOI: 10.1191/1358863x02vm412ra]
37 **Lane JT**. Microalbuminuria as a marker of cardiovascular and renal risk in type 2 diabetes mellitus: a temporal perspective. *Am J Physiol Renal Physiol* 2004; **286**: F442-F450 [PMID: 14761931 DOI: 10.1152/ajprenal.00247.2003]
38 **Basi S**, Lewis JB. Microalbuminuria as a target to improve cardiovascular and renal outcomes. *Am J Kidney Dis* 2006; **47**: 927-946 [PMID: 16731288 DOI: 10.1053/j.ajkd.2006.02.182]
39 **Adler AI**, Stevens RJ, Manley SE, Bilous RW, Cull CA, Holman RR. Development and progression of nephropathy in type 2 diabetes: the United Kingdom Prospective Diabetes Study (UKPDS 64). *Kidney Int* 2003; **63**: 225-232 [PMID: 12472787 DOI: 10.1046/j.1523-1755.2003.00712.x]
40 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]
41 **Writing Team for the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group**. Sustained effect of intensive treatment of type 1 diabetes mellitus on development and progression of diabetic nephropathy: the Epidemiology of Diabetes Interventions and Complications (EDIC) study. *JAMA* 2003; **290**: 2159-2167 [PMID: 14570951 DOI: 10.1001/jama.290.16.2159]
42 **de Boer IH**, Sun W, Cleary PA, Lachin JM, Molitch ME, Steffes MW, Zinman B. Intensive diabetes therapy and glomerular filtration rate in type 1 diabetes. *N Engl J Med* 2011; **365**: 2366-2376 [PMID: 22077236 DOI: 10.1056/NEJMoa1111732]
43 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]
44 **Ohkubo Y**, Kishikawa H, Araki E, Miyata T, Isami S, Motoyoshi S, Kojima Y, Furuyoshi N, Shichiri M. Intensive insulin therapy prevents the progression of diabetic microvascular complications in Japanese patients with non-insulin-dependent diabetes mellitus: a randomized prospective 6-year study. *Diabetes Res Clin Pract* 1995; **28**: 103-117 [PMID: 7587918 DOI: 10.1016/0168-8227(95)01064-K]
45 **Ismail-Beigi F**, Craven T, Banerji MA, Basile J, Calles J, Cohen RM, Cuddihy R, Cushman WC, Genuth S, Grimm RH, Hamilton BP, Hoogwerf B, Karl D, Katz L, Krikorian A, O'Connor P, Pop-Busui R, Schubart U, Simmons D, Taylor H, Thomas A, Weiss D, Hramiak I. Effect of intensive treatment of hyperglycaemia on microvascular outcomes in type 2 diabetes: an analysis of the ACCORD randomised trial. *Lancet* 2010; **376**: 419-430 [PMID: 20594588 DOI: 10.1016/S0140-6736(10)60576-4]
46 **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, Bompoint 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]
47 **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]
48 **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]
49 **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]
50 Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. UK Prospective Diabetes Study Group. *BMJ* 1998; **317**: 703-713 [PMID: 9732337 DOI: 10.1136/bmj.317.7160.703]
51 **Patel A**, MacMahon S, Chalmers J, Neal B, Woodward M, Billot L, Harrap S, Poulter N, Marre M, Cooper M, Glasziou P, Grobbee DE, Hamet P, Heller S, Liu LS, Mancia G, Mogensen CE, Pan CY, Rodgers A, Williams B. Effects of a fixed combination of perindopril and indapamide on macrovascular and microvascular outcomes in patients with type 2 diabetes mellitus (the ADVANCE trial): a randomised controlled trial. *Lancet* 2007; **370**: 829-840 [PMID: 17765963 DOI: 10.1016/S0140-6736(07)61303-8]
52 **Ninomiya T**, Perkovic V, de Galan BE, Zoungas S, Pillai A, Jardine M, Patel A, Cass A, Neal B, Poulter N, Mogensen CE, Cooper M, Marre M, Williams B, Hamet P, Mancia G, Woodward M, Macmahon S, Chalmers J. Albuminuria and kidney function independently predict cardiovascular and renal outcomes in diabetes. *J Am Soc Nephrol* 2009; **20**: 1813-1821 [PMID: 19443635 DOI: 10.1681/ASN.2008121270]
53 **James PA**, Oparil S, Carter BL, Cushman WC, Dennison-Himmelfarb C, Handler J, Lackland DT, LeFevre ML, MacKenzie TD, Ogedegbe O, Smith SC, Svetkey LP, Taler SJ, Townsend RR, Wright JT, Narva AS, Ortiz E. 2014 evidence-based guideline for the management of high blood pressure in adults: report from the panel members appointed to the Eighth Joint National Committee (JNC 8). *JAMA* 2014; **311**: 507-520 [PMID: 24352797 DOI: 10.1001/jama.2013.284427]
54 **Koya D**, Araki S, and Haneda M. Management of diabetes kidney disease. *J Diabetes Invest* 2011; **2**: 248-254 [DOI: 10.1111/j.2040-1124.2011.00112.x]
55 **Fineberg D**, Jandeleit-Dahm KA, Cooper ME. Diabetic nephropathy: diagnosis and treatment. *Nat Rev Endocrinol* 2013; **9**: 713-723 [PMID: 24100266 DOI: 10.1038/nrendo.2013.184]
56 **Ruggenenti P**, Fassi A, Ilieva AP, Bruno S, Iliev IP, Brusegan

V, Rubis N, Gherardi G, Arnoldi F, Ganeva M, Ene-Iordache B, Gaspari F, Perna A, Bossi A, Trevisan R, Dodesini AR, Remuzzi G. Preventing microalbuminuria in type 2 diabetes. *N Engl J Med* 2004; **351**: 1941-1951 [PMID: 15516697 DOI: 10.1056/NEJMoa042167]

57 **Parving HH**, Lehnert H, Bröchner-Mortensen J, Gomis R, Andersen S, Arner P. The effect of irbesartan on the development of diabetic nephropathy in patients with type 2 diabetes. *N Engl J Med* 2001; **345**: 870-878 [PMID: 11565519 DOI: 10.1056/NEJMoa011489]

58 **Viberti G**, Wheeldon NM. Microalbuminuria reduction with valsartan in patients with type 2 diabetes mellitus: a blood pressure-independent effect. *Circulation* 2002; **106**: 672-678 [PMID: 12163426 DOI: 10.1161/01.CIR.0000024416.33113.0A]

59 **Barnett AH**, Bain SC, Bouter P, Karlberg B, Madsbad S, Jervell J, Mustonen J. Angiotensin-receptor blockade versus converting-enzyme inhibition in type 2 diabetes and nephropathy. *N Engl J Med* 2004; **351**: 1952-1961 [PMID: 15516696 DOI: 10.1056/NEJMoa042274]

60 **Makino H**, Haneda M, Babazono T, Moriya T, Ito S, Iwamoto Y, Kawamori R, Takeuchi M, Katayama S. Prevention of transition from incipient to overt nephropathy with telmisartan in patients with type 2 diabetes. *Diabetes Care* 2007; **30**: 1577-1578 [PMID: 17389334 DOI: 10.2337/dc06-1998]

61 **Lewis EJ**, Hunsicker LG, Clarke WR, Berl T, Pohl MA, Lewis JB, Ritz E, Atkins RC, Rohde R, Raz I. Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. *N Engl J Med* 2001; **345**: 851-860 [PMID: 11565517 DOI: 10.1056/NEJMoa011303]

62 **Brenner BM**, Cooper ME, de Zeeuw D, Keane WF, Mitch WE, Parving HH, Remuzzi G, Snapinn SM, Zhang Z, Shahinfar S. Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *N Engl J Med* 2001; **345**: 861-869 [PMID: 11565518 DOI: 10.1056/NEJMoa011161]

63 **Parving HH**, Persson F, Lewis JB, Lewis EJ, Hollenberg NK. Aliskiren combined with losartan in type 2 diabetes and nephropathy. *N Engl J Med* 2008; **358**: 2433-2446 [PMID: 18525041 DOI: 10.1056/NEJMoa0708379]

64 Randomised placebo-controlled trial of lisinopril in normotensive patients with insulin-dependent diabetes and normoalbuminuria or microalbuminuria. The EUCLID Study Group. *Lancet* 1997; **349**: 1787-1792 [PMID: 9269212 DOI: 10.1016/S0140-6736(96)10244-0]

65 **Katayama S**, Kikkawa R, Isogai S, Sasaki N, Matsuura N, Tajima N, Urakami T, Uchigata Y, Ohashi Y. Effect of captopril or imidapril on the progression of diabetic nephropathy in Japanese with type 1 diabetes mellitus: a randomized controlled study (JAPAN-IDDM). *Diabetes Res Clin Pract* 2002; **55**: 113-121 [PMID: 11796177 DOI: 10.1016/S0168-8227(01)00289-3]

66 **Lewis EJ**, Hunsicker LG, Bain RP, Rohde RD. The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. The Collaborative Study Group. *N Engl J Med* 1993; **329**: 1456-1462 [PMID: 8413456 DOI: 10.1056/NEJM199311113292004]

67 **Yusuf S**, Teo KK, Pogue J, Dyal L, Copland I, Schumacher H, Dagenais G, Sleight P, Anderson C. Telmisartan, ramipril, or both in patients at high risk for vascular events. *N Engl J Med* 2008; **358**: 1547-1559 [PMID: 18378520 DOI: 10.1056/NEJMoa0801317]

68 **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]

69 **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]

70 **Kang YS**, Ko GJ, Lee MH, Song HK, Han SY, Han KH, Kim HK, Han JY, Cha DR. Effect of eplerenone, enalapril and their combination treatment on diabetic nephropathy in type II diabetic rats. *Nephrol Dial Transplant* 2009; **24**: 73-84 [PMID: 18682491 DOI: 10.1093/ndt/gfn448]

71 **Rachmani R**, Slavachevsky I, Amit M, Levi Z, Kedar Y, Berla M, Ravid M. The effect of spironolactone, cilazapril and their combination on albuminuria in patients with hypertension and diabetic nephropathy is independent of blood pressure reduction: a randomized controlled study. *Diabet Med* 2004; **21**: 471-475 [PMID: 15089793 DOI: 10.1111/j.1464-5491.2004.01194.x]

72 **Rossing K**, Schjoedt KJ, Smidt UM, Boomsma F, Parving HH. Beneficial effects of adding spironolactone to recommended antihypertensive treatment in diabetic nephropathy: a randomized, double-masked, cross-over study. *Diabetes Care* 2005; **28**: 2106-2112 [PMID: 16123474 DOI: 10.2337/diacare.28.9.2106]

73 **Schjoedt KJ**, Rossing K, Juhl TR, Boomsma F, Tarnow L, Rossing P, Parving HH. Beneficial impact of spironolactone on nephrotic range albuminuria in diabetic nephropathy. *Kidney Int* 2006; **70**: 536-542 [PMID: 16775595]

74 **Bianchi S**, Bigazzi R, Campese VM. Antagonists of aldosterone and proteinuria in patients with CKD: an uncontrolled pilot study. *Am J Kidney Dis* 2005; **46**: 45-51 [PMID: 15983956 DOI: 10.1053/j.ajkd.2005.03.007]

75 **Mehdi UF**, Adams-Huet B, Raskin P, Vega GL, Toto RD. Addition of angiotensin receptor blockade or mineralocorticoid antagonism to maximal angiotensin-converting enzyme inhibition in diabetic nephropathy. *J Am Soc Nephrol* 2009; **20**: 2641-2650 [PMID: 19926893 DOI: 10.1681/ASN.2009070737]

76 **Parving HH**, Brenner BM, McMurray JJ, de Zeeuw D, Haffner SM, Solomon SD, Chaturvedi N, Persson F, Desai AS, Nicolaides 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]

77 **Bakris GL**, Toto RD, McCullough PA, Rocha R, Purkayastha D, Davis P. Effects of different ACE inhibitor combinations on albuminuria: results of the GUARD study. *Kidney Int* 2008; **73**: 1303-1309 [PMID: 18354383 DOI: 10.1038/ki.2008.102]

78 **Jamerson K**, Weber MA, Bakris GL, Dahlöf B, Pitt B, Shi V, Hester A, Gupte J, Gatlin M, Velazquez EJ. Benazepril plus amlodipine or hydrochlorothiazide for hypertension in high-risk patients. *N Engl J Med* 2008; **359**: 2417-2428 [PMID: 19052124 DOI: 10.1056/NEJMoa0806182]

79 **Bakris GL**, Sarafidis PA, Weir MR, Dahlöf B, Pitt B, Jamerson K, Velazquez EJ, Staikos-Byrne L, Kelly RY, Shi V, Chiang YT, Weber MA. Renal outcomes with different fixed-dose combination therapies in patients with hypertension at high risk for cardiovascular events (ACCOMPLISH): a prespecified secondary analysis of a randomised controlled trial. *Lancet* 2010; **375**: 1173-1181 [PMID: 20170948 DOI: 10.1016/S0140-6736(09)62100-0]

80 **Koya D**, Campese VM. Statin use in patients with diabetes and kidney disease: the Japanese experience. *J Atheroscler Thromb* 2013; **20**: 407-424 [PMID: 23518468 DOI: 10.5551/jat.16261]

81 **Tonelli M**, Muntner P, Lloyd A, Manns BJ, Klarenbach S, Pannu N, James MT, Hemmelgarn BR. Risk of coronary events in people with chronic kidney disease compared with those with diabetes: a population-level cohort study. *Lancet* 2012; **380**: 807-814 [PMID: 22717317 DOI: 10.1016/S0140-6736(12)60572-8]

82 **Shepherd J**, Kastelein JJ, Bittner V, Deedwania P, Breazna A, Dobson S, Wilson DJ, Zuckerman A, Wenger NK. Intensive lipid lowering with atorvastatin in patients with coronary heart disease and chronic kidney disease: the TNT (Treating to New Targets) study. *J Am Coll Cardiol* 2008; **51**: 1448-1454 [PMID: 18402899 DOI: 10.1016/j.jacc.2007.11.072]

83 **Koren MJ**, Davidson MH, Wilson DJ, Fayyad RS, Zuckerman A, Reed DP. Focused atorvastatin therapy in managed-care patients with coronary heart disease and CKD. *Am J Kidney Dis* 2009; **53**: 741-750 [PMID: 19216014 DOI: 10.1053/j.ajkd.2008.11.025]

84 **Tonelli M**, Moyé L, Sacks FM, Cole T, Curhan GC. Effect of pravastatin on loss of renal function in people with moderate chronic renal insufficiency and cardiovascular disease. *J Am Soc Nephrol* 2003; **14**: 1605-1613 [PMID: 12761262 DOI: 10.1097/01.ASN.0000068461.45784.2F]

85 **Tonelli M**, Isles C, Craven T, Tonkin A, Pfeffer MA, Shepherd J, Sacks FM, Furberg C, Cobbe SM, Simes J, West M, Packard C, Curhan GC. Effect of pravastatin on rate of kidney function loss in people with or at risk for coronary disease. *Circulation* 2005; **112**: 171-178 [PMID: 15998677 DOI: 10.1161/CIRCULATIONAHA.104.517565]

86 **Vidt DG**, Ridker PM, Monyak JT, Schreiber MJ, Cressman MD. Longitudinal assessment of estimated glomerular filtration rate in apparently healthy adults: a post hoc analysis from the JUPITER study (justification for the use of statins in prevention: an intervention trial evaluating rosuvastatin). *Clin Ther* 2011; **33**: 717-725 [PMID: 21704236 DOI: 10.1016/j.clinthera.2011.05.004]

87 **Sandhu S**, Wiebe N, Fried LF, Tonelli M. Statins for improving renal outcomes: a meta-analysis. *J Am Soc Nephrol* 2006; **17**: 2006-2016 [PMID: 16762986 DOI: 10.1681/ASN.2006010012]

88 **Takagi H**, Umemoto T. A meta-analysis of randomized trials for effects of atorvastatin on renal function in chronic kidney disease. *Int J Cardiol* 2011; **152**: 242-244 [PMID: 21764159 DOI: 10.1016/j.ijcard.2011.06.107]

89 **Kimura S**, Inoguchi T, Yokomizo H, Maeda Y, Sonoda N, Takayanagi R. Randomized comparison of pitavastatin and pravastatin treatment on the reduction of urinary albumin in patients with type 2 diabetic nephropathy. *Diabetes Obes Metab* 2012; **14**: 666-669 [PMID: 22268518 DOI: 10.1111/j.1463-1326.2012.01566.x]

90 **Colhoun HM**, Betteridge DJ, Durrington PN, Hitman GA, Neil HA, Livingstone SJ, Charlton-Menys V, DeMicco DA, Fuller JH. Effects of atorvastatin on kidney outcomes and cardiovascular disease in patients with diabetes: an analysis from the Collaborative Atorvastatin Diabetes Study (CARDS). *Am J Kidney Dis* 2009; **54**: 810-819 [PMID: 19540640 DOI: 10.1053/j.ajkd.2009.03.022]

91 **Jun M**, Zhu B, Tonelli M, Jardine MJ, Patel A, Neal B, Liyanage T, Keech A, Cass A, Perkovic V. Effects of fibrates in kidney disease: a systematic review and meta-analysis. *J Am Coll Cardiol* 2012; **60**: 2061-2071 [PMID: 23083786 DOI: 10.1016/j.jacc.2012.07.049]

92 **Palmer SC**, Craig JC, Navaneethan SD, Tonelli M, Pellegrini F, Strippoli GF. Benefits and harms of statin therapy for persons with chronic kidney disease: a systematic review and meta-analysis. *Ann Intern Med* 2012; **157**: 263-275 [PMID: 22910937 DOI: 10.7326/0003-4819-157-4-201208210-00007]

93 **Haslinger-Löffler B**. Multiple effects of HMG-CoA reductase inhibitors (statins) besides their lipid-lowering function. *Kidney Int* 2008; **74**: 553-555 [PMID: 18709024 DOI: 10.1038/ki.2008.323]

94 **Balakumar P**, Kadian S, Mahadevan N. Are PPAR alpha agonists a rational therapeutic strategy for preventing abnormalities of the diabetic kidney? *Pharmacol Res* 2012; **65**: 430-436 [PMID: 22285932 DOI: 10.1016/j.phrs.2012.01.004]

95 **Gaede P**, Vedel P, Larsen N, Jensen GV, Parving HH, Pedersen O. Multifactorial intervention and cardiovascular disease in patients with type 2 diabetes. *N Engl J Med* 2003; **348**: 383-393 [PMID: 12556541 DOI: 10.1056/NEJMoa021778]

96 **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]

97 **Tu ST**, Chang SJ, Chen JF, Tien KJ, Hsiao JY, Chen HC, Hsieh MC. Prevention of diabetic nephropathy by tight target control in an asian population with type 2 diabetes mellitus: a 4-year prospective analysis. *Arch Intern Med* 2010; **170**: 155-161 [PMID: 20101010 DOI: 10.1001/archinternmed.2009.471]

98 **Araki S**, Haneda M, Sugimoto T, Isono M, Isshiki K, Kashiwagi A, Koya D. Factors associated with frequent remission of microalbuminuria in patients with type 2 diabetes. *Diabetes* 2005; **54**: 2983-2987 [PMID: 16186402 DOI: 10.2337/diabetes.54.10.2983]

99 **Gaede P**, Tarnow L, Vedel P, Parving HH, Pedersen O. Remission to normoalbuminuria during multifactorial treatment preserves kidney function in patients with type 2 diabetes and microalbuminuria. *Nephrol Dial Transplant* 2004; **19**: 2784-2788 [PMID: 15328385 DOI: 10.1093/ndt/gfh470]

100 **de Galan BE**, Perkovic V, Ninomiya T, Pillai A, Patel A, Cass A, Neal B, Poulter N, Harrap S, Mogensen CE, Cooper M, Marre M, Williams B, Hamet P, Mancia G, Woodward M, Glasziou P, Grobbee DE, MacMahon S, Chalmers J. Lowering blood pressure reduces renal events in type 2 diabetes. *J Am Soc Nephrol* 2009; **20**: 883-892 [PMID: 19225038 DOI: 10.1681/ASN.2008070667]

101 **Araki S**, Haneda M, Koya D, Hidaka H, Sugimoto T, Isono M, Isshiki K, Chin-Kanasaki M, Uzu T, Kashiwagi A. Reduction in microalbuminuria as an integrated indicator for renal and cardiovascular risk reduction in patients with type 2 diabetes. *Diabetes* 2007; **56**: 1727-1730 [PMID: 17360976 DOI: 10.2337/db06-1646]

102 **Lindeman RD**, Tobin J, Shock NW. Longitudinal studies on the rate of decline in renal function with age. *J Am Geriatr Soc* 1985; **33**: 278-285 [PMID: 3989190]

103 **Li YC**. Vitamin D: roles in renal and cardiovascular protection. *Curr Opin Nephrol Hypertens* 2012; **21**: 72-79 [PMID: 22143249 DOI: 10.1097/MNH.0b013e32834de4ee]

104 **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]

105 **Kim MJ**, Frankel AH, Donaldson M, Darch SJ, Pusey CD, Hill PD, Mayr M, Tam FW. Oral cholecalciferol decreases albuminuria and urinary TGF-β1 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]

106 **Hovind P**, Rossing P, Tarnow L, Johnson RJ, Parving HH. Serum uric acid as a predictor for development of diabetic nephropathy in type 1 diabetes: an inception cohort study. *Diabetes* 2009; **58**: 1668-1671 [PMID: 19411615 DOI: 10.2337/db09-0014]

107 **Jalal DI**, Rivard CJ, Johnson RJ, Maahs DM, McFann K, Rewers M, Snell-Bergeon JK. Serum uric acid levels predict the development of albuminuria over 6 years in patients with type 1 diabetes: findings from the Coronary Artery Calcification in Type 1 Diabetes study. *Nephrol Dial Transplant* 2010; **25**: 1865-1869 [PMID: 20064950 DOI: 10.1093/ndt/gfp740]

108 **Ficociello LH**, Rosolowsky ET, Niewczas MA, Maselli NJ, Weinberg JM, Aschengrau A, Eckfeldt JH, Stanton RC, Galecki AT, Doria A, Warram JH, Krolewski AS. High-normal serum uric acid increases risk of early progressive renal function loss in type 1 diabetes: results of a 6-year follow-up. *Diabetes Care* 2010; **33**: 1337-1343 [PMID: 20332356 DOI: 10.2337/dc10-0227]

109 **Miao Y**, Ottenbros SA, Laverman GD, Brenner BM, Cooper ME, Parving HH, Grobbee DE, Shahinfar S, de Zeeuw D, Lambers Heerspink HJ. Effect of a reduction in uric acid on renal outcomes during losartan treatment: a post hoc analysis of the reduction of endpoints in non-insulin-dependent diabetes mellitus with the Angiotensin II Antagonist Losartan Trial. *Hypertension* 2011; **58**: 2-7 [PMID: 21632472 DOI: 10.1161/HYPERTENSIONAHA.111.171488]

110 **Kim W**, Egan JM. The role of incretins in glucose homeostasis and diabetes treatment. *Pharmacol Rev* 2008; **60**: 470-512 [PMID: 19074620 DOI: 10.1124/pr.108.000604]

111 **Gallwitz B**. The evolving place of incretin-based therapies in type 2 diabetes. *Pediatr Nephrol* 2010; **25**: 1207-1217 [PMID: 20130920 DOI: 10.1007/s00467-009-1435-z]

112 **Neumiller JJ**. Clinical pharmacology of incretin therapies for type 2 diabetes mellitus: implications for treatment. *Clin Ther* 2011; **33**: 528-576 [PMID: 21665041 DOI: 10.1016/j.clinthera.2011.04.024]

113 **Bullock BP**, Heller RS, Habener JF. Tissue distribution of messenger ribonucleic acid encoding the rat glucagon-like peptide-1 receptor. *Endocrinology* 1996; **137**: 2968-2978 [PMID: 8770921]

114 **Panchapakesan U**, Mather A, Pollock C. Role of GLP-1 and DPP-4 in diabetic nephropathy and cardiovascular disease. *Clin Sci* (Lond) 2013; **124**: 17-26 [PMID: 22963445 DOI: 10.1042/CS20120167]

115 **Mima A**, Hiraoka-Yamomoto J, Li Q, Kitada M, Li C, Geraldes P, Matsumoto M, Mizutani K, Park K, Cahill C, Nishikawa S, Rask-Madsen C, King GL. Protective effects of GLP-1 on glomerular endothelium and its inhibition by PKCβ activation in diabetes. *Diabetes* 2012; **61**: 2967-2979 [PMID: 22826029 DOI: 10.2337/db11-1824]

116 **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]

117 **Fujita H**, Morii T, Fujishima H, Sato T, Shimizu T, Hosoba M, Tsukiyama K, Narita T, Takahashi T, Drucker DJ, Seino Y, Yamada Y. The protective roles of GLP-1R signaling in diabetic nephropathy: possible mechanism and therapeutic potential. *Kidney Int* 2014; **85**: 579-589 [PMID: 24152968 DOI: 10.1038/ki.2013.427]

118 **Li W**, Cui M, Wei Y, Kong X, Tang L, Xu D. Inhibition of the expression of TGF-β1 and CTGF in human mesangial cells by exendin-4, a glucagon-like peptide-1 receptor agonist. *Cell Physiol Biochem* 2012; **30**: 749-757 [PMID: 22890152 DOI: 10.1159/000341454]

119 **Schlatter P**, Beglinger C, Drewe J, Gutmann H. Glucagon-like peptide 1 receptor expression in primary porcine proximal tubular cells. *Regul Pept* 2007; **141**: 120-128 [PMID: 17276524 DOI: 10.1016/j.regpep.2006.12.016]

120 **Hendarto H**, Inoguchi T, Maeda Y, Ikeda N, Zheng J, Takei R, Yokomizo H, Hirata E, Sonoda N, Takayanagi R. GLP-1 analog liraglutide protects against oxidative stress and albuminuria in streptozotocin-induced diabetic rats via protein kinase A-mediated inhibition of renal NAD(P)H oxidases. *Metabolism* 2012; **61**: 1422-1434 [PMID: 22554832 DOI: 10.1016/j.metabol.2012.03.002]

121 **Hirata K**, Kume S, Araki S, Sakaguchi M, Chin-Kanasaki M, Isshiki K, Sugimoto T, Nishiyama A, Koya D, Haneda M, Kashiwagi A, Uzu T. Exendin-4 has an anti-hypertensive effect in salt-sensitive mice model. *Biochem Biophys Res Commun* 2009; **380**: 44-49 [PMID: 19150338 DOI: 10.1016/j.bbrc.2009.01.003]

122 **Mentlein R**. Dipeptidyl-peptidase IV (CD26)--role in the inactivation of regulatory peptides. *Regul Pept* 1999; **85**: 9-24 [PMID: 10588446 DOI: 10.1016/S0167-0115(99)00089-0]

123 **Sun AL**, Deng JT, Guan GJ, Chen SH, Liu YT, Cheng J, Li ZW, Zhuang XH, Sun FD, Deng HP. Dipeptidyl peptidase-IV is a potential molecular biomarker in diabetic kidney disease. *Diab Vasc Dis Res* 2012; **9**: 301-308 [PMID: 22388283 DOI: 10.1177/1479164111434318]

124 **Yang J**, Campitelli J, Hu G, Lin Y, Luo J, Xue C. Increase in DPP-IV in the intestine, liver and kidney of the rat treated with high fat diet and streptozotocin. *Life Sci* 2007; **81**: 272-279 [PMID: 17583752 DOI: 10.1016/j.lfs.2007.04.040]

125 **Ansorge S**, Nordhoff K, Bank U, Heimburg A, Julius H, Breyer D, Thielitz A, Reinhold D, Täger M. Novel aspects of cellular action of dipeptidyl peptidase IV/CD26. *Biol Chem* 2011; **392**: 153-168 [PMID: 21194362 DOI: 10.1515/bc.2011.008]

126 **Hattori S**. Sitagliptin reduces albuminuria in patients with type 2 diabetes. *Endocr J* 2011; **58**: 69-73 [PMID: 21206136 DOI: 10.1507/endocrj.K10E-382]

127 **Harashima SI**, Ogura M, Tanaka D, Fukushima T, Wang Y, Koizumi T, Aono M, Murata Y, Seike M, Inagaki N. Sitagliptin add-on to low dosage sulphonylureas: efficacy and safety of combination therapy on glycaemic control and insulin secretion capacity in type 2 diabetes. *Int J Clin Pract* 2012; **66**: 465-476 [PMID: 22512606 DOI: 10.1111/j.1742-1241.2012.02903.x]

128 **Zhang H**, Zhang X, Hu C, Lu W. Exenatide reduces urinary transforming growth factor-β1 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]

129 **Groop PH**, Cooper ME, Perkovic V, Emser A, Woerle HJ, von Eynatten M. Linagliptin lowers albuminuria on top of recommended standard treatment in patients with type 2 diabetes and renal dysfunction. *Diabetes Care* 2013; **36**: 3460-3468 [PMID: 24026560 DOI: 10.2337/dc13-0323]

130 **Jerums G**, Panagiotopoulos S, Premaratne E, MacIsaac RJ. Integrating albuminuria and GFR in the assessment of diabetic nephropathy. *Nat Rev Nephrol* 2009; **5**: 397-406 [PMID: 19556994 DOI: 10.1038/nrneph.2009.91]

131 **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]

132 **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]

133 **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]

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REVIEW

Adipokines as a novel link between obesity and atherosclerosis

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Abstract

The traditional perception of adipose tissue as a storage organ of fatty acids has been replaced by the notion that adipose tissue is an active endocrine organ, releasing various adipokines that are involved in the pathogenesis of obesity-related metabolic disturbances. Obesity is a well-known risk factor for atherosclerosis, and accelerates atherosclerosis by many mechanisms such as increase in blood pressure and glucose level, abnormal lipid profiles, and systemic inflammation. Furthermore, growing evidence suggests that some adipokines directly mediate the process of atherosclerosis by influencing the function of endothelial cells, arterial smooth muscle cells, and macrophages in vessel walls. In obese patients, the secretion and coordination of such adipokines is abnormal, and the secretion of specific adipokines increases or decreases. Accordingly, the discovery of new adipokines and elucidation of their functions might lead to a new treatment strategy for metabolic disorders related to obesity, including cardiovascular diseases.

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Key words: Adipose tissue; Adipokine; Obesity; Atherosclerosis; Inflammation

Core tip: This review summarizes recent laboratory and clinical studies on the influence of various adipokines, including adiponectin, resistin, adipocyte fatty acid binding protein, omentin-1, and chemerin, on the development of atherosclerosis.

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INTRODUCTION

Obesity is an important risk factor for atherosclerosis, but the underlying mechanism for this association is poorly understood. Adipose tissue was considered to be a store of surplus energy, but is now recognized as an independent and active endocrine organ. Various adipokines, such as leptin (a protein secreted by fat cells), tumor necrosis factor-α (TNF-α), resistin, and adiponectin significantly affect obesity-related metabolic diseases by controlling fat metabolism, energy homeostasis, and insulin sensitivity[1]. Independent of their effects on glucose and fat metabolism, some adipokines have been regarded recently as direct links between obesity and atherosclerosis because of their influence on the function of endothelial cells, arterial smooth muscle cells, and macrophages in vessel walls[2] (Figure 1). The identification of a novel adipokine that regulates the atherosclerotic process might provide new opportunities for developing more effective approaches for preventing cardiovascular disease. This review will focus on adipokines that mediate obesity and atherosclerosis, including adiponectin, resistin, adipocyte fatty acid binding protein (A-FABP), omentin-1, and chemerin.

ADIPONECTIN

Adiponectin was the first 30-kDa protein cloned from fat

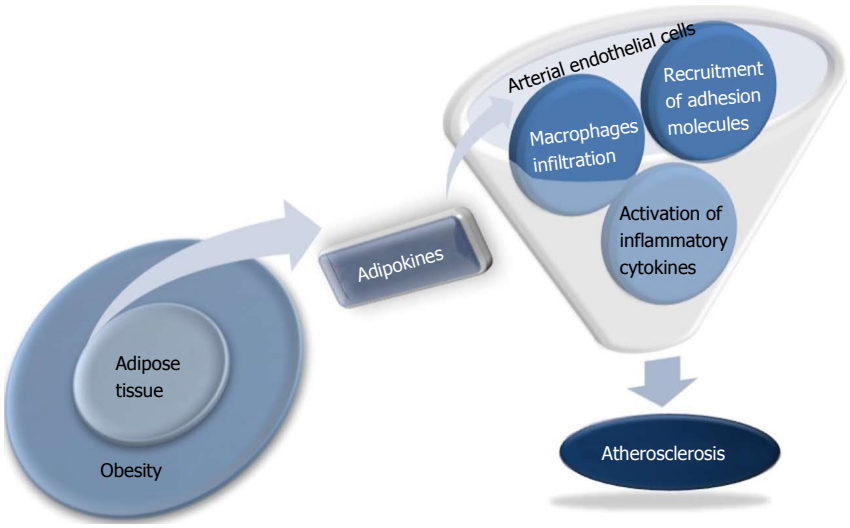

Figure 1 Novel function of adipokines as a direct link between obesity and atherosclerosis.

tissues[3]. Adiponectin is a metabolically active adipokine that is inversely associated with obesity, insulin resistance, and atherosclerosis[4,5]. Adiponectin promotes fatty acid oxidation through the phosphorylation of 5-AMP-activated protein kinase (AMPK), thereby stimulating acetyl-CoA carboxylase. The adiponectin receptors AdipoR1 and AdipoR2 are responsible for adiponectin signaling and biological function. Yamauchi *et al*[6] reported that insulin resistance occurred in AdipoR1/R2 knockout mice, but when AdipoR1 or AdipoR2 were overexpressed in the liver by using adenovirus, glucose metabolism improved in terms of increase in AMPK vitality and peroxisome proliferator-activated receptors α expression. Adiponectin is a metabolically active adipokine which has anti-inflammatory, antiatherogenic, and antidiabetic properties[7] and is therefore inversely associated with obesity, insulin resistance, and atherosclerosis. Hypoadiponectinemia has been established as an independent risk factor for type 2 diabetes and cardiovascular disease (CVD)[8]. We previously showed that, after adjusting for age, sex, obesity, history of impaired fasting glucose or impaired glucose tolerance, hypertension, and dyslipidemia, lower baseline serum adiponectin concentrations are associated significantly with the development of type 2 diabetes and metabolic syndrome[9]. On the other side, the Health Professionals Follow-Up Study showed that high plasma adiponectin levels were associated with a lower risk of myocardial infarction in men during 6 years of follow-up studies[10].

Experimental studies have shown that adiponectin plays a protective role against the development of inflammation and atherosclerosis. Ouchi *et al*[11] demonstrated that adiponectin specifically suppressed TNF-α-induced nuclear factor κ light chain enhancer of activated B cells (NF-κB) activation in human aortic endothelial cells (HAECs) through a cAMP-dependent pathway. Furthermore, adiponectin suppressed TNF-α-mediated induction of adhesion molecule expression in HAECs. Recently, we reported that serum adiponectin levels had a significant negative correlation with vascular inflammation as indicated by the mean target to background ratio (TBR), suggesting a cardio-protective effect of adiponectin[12].

RESISTIN

Resistin was originally discovered as an adipokine with a possible link between obesity and insulin resistance in rodents[13]. In contrast to rodents, human resistin is expressed primarily in inflammatory cells and has been shown to be involved in obesity-related subclinical inflammation, atherosclerosis, and CVD[14]. Reilly *et al*[15] showed that circulating resistin levels are correlated with inflammation markers and are predictive of coronary atherosclerosis, as measured by coronary artery calcification scores, independent of C-reactive protein. Kawanami *et al*[16] found that resistin induces the expression of adhesion molecules, such as vascular cellular adhesion molecule-1 and intercellular adhesion molecule-1 and that adiponectin inhibit the effect of resistin in vascular endothelial cells. Lee *et al*[17] observed that resistin promotes foam cell formation *via* the dysregulation of scavenger receptors macrophages. In men with acute myocardial infarction, a multivariate model revealed that obesity and C-reactive protein were independent variables associated with higher resistin levels[18]. In a cross-sectional study of 3193 Chinese subjects, resistin was more significantly associated with fibrinolytic and inflammatory markers than with obesity or insulin resistance[19]. Moreover, Weikert *et al*[20] reported that individuals in the highest quartile of resistin levels had a significantly increased risk of myocardial infarction compared with those in the lowest quartile of resistin levels after adjustment for cardiovascular risk factors, including C-reactive protein (RR = 2.09; 95%CI: 1.01-4.31) in 26490 middle-aged subjects. Among 397 South Korean patients with acute myocardial infarction, high resistin level was an significant predictor for all-cause mortality, independent of other confounding risk factors[21]. We also showed that serum resistin levels were positively correlated with vascular inflammation mea-

sured using ^{18}F-fluoro-deoxyglucose positron emission tomography[12]. These studies suggest that resistin may represent a novel linkage of metabolic signals, inflammation, and atherosclerosis.

ADIPOCYTE FATTY ACID BINDING PROTEIN

A-FABP is a cytoplasmic protein that combines with saturated and unsaturated fatty acids to control the distribution of fatty acids in various inflammatory response and metabolic pathways[22]. Since Xu *et al*[23] established that the serum concentration of A-FABP, which is synthesized in cytoplasm and secreted into serum, is significantly correlated with components of metabolic syndrome, the role of A-FABP in metabolic syndrome has been studied with renewed interest. Uysal *et al*[24] proved through an oral glucose tolerance test that insulin sensitivity was increased in A-FABP knock out ob/ob mice compared with control mice. In prospective studies, circulating A-FABP has been shown to predict the development of metabolic syndrome and type 2 diabetes independent of adiposity and insulin resistance[25,26].

A-FABP has been shown to be a major mediator of vulnerable plaque formation in various animal and *in vitro* studies. The survival rates of apoE$^{-/-}$ mice null for both A-FABP and mal1 were significantly higher than apoE$^{-/-}$ control mice, primarily because of increased stability of atherosclerotic plaques[27]. In macrophage cell lines, adenovirus-mediated over-expression of A-FABP directly induced foam cell formation by increasing intracellular lipid accumulation, which is an essential step in the formation of atherosclerotic plaques[28]. In contrast, A-FABP$^{-/-}$ macrophages displayed significantly decreased intracellular cholesterol ester accumulation *in vitro*[29] and suppressed production of inflammatory cytokines, such as TNF-α, monocyte chemoattractant protein-1, and interleukin (IL)-6, compared with wild-type controls[30]. Furthermore, Furuhashi *et al*[31] reported that an orally active small molecule inhibitor of A-FABP was an effective therapeutic agent against severe atherosclerosis in mouse models. Recently, a few clinical studies have shown that circulating A-FABP levels are closely related to the development of atherosclerosis in humans. In Korean subjects in whom coronary angiograms were performed for evaluation of chest pain, serum A-FABP levels increased as the number of stenotic coronary arteries increased[32]. Serum A-FABP was shown to be independently associated with carotid intima-media thickness (IMT) in Chinese women after adjusting for other risk factors, including age, obesity, and blood pressure[33]. In patients with coronary artery disease recruited to undergo elective percutaneous coronary intervention, Miyoshi *et al*[34] showed that increased serum A-FABP levels were significantly associated with a greater coronary plaque burden as quantified by intravascular ultrasound. After adjusting for other cardiovascular risk factor in South Korean men without cardiovascular disease or diabetes, we reported that circulating A-FABP levels were independently associated with vascular inflammation as measured by maximum TBR values[35], suggesting A-FABP as a promising key link between different metabolic pathways of adiposity and inflammation.

OMENTIN-1

Omentin is a visceral fat-specific adipokine discovered through expressed sequence tag analysis[36] that has paracrine and autocrine roles in improving insulin sensitivity. Yang *et al*[37] demonstrated that the addition of recombinant omentin stimulated glucose uptake in human adipocytes *via* the activation of Akt phosphorylation. Recent studies showed that omentin increased insulin signal transduction and that it was significantly negatively correlated with metabolic risk factors, including obesity and hyperglycemia, thereby suggesting a beneficial role in energy homeostasis[38-40]. In human clinical studies, it has been suggested that serum omentin-1 levels were significantly decreased in metabolically unhealthy states, such as metabolic syndrome, types 2 diabetes mellitus, and polycystic ovarian syndrome[38-40].

Expression of the omentin gene in interstitial and endothelial cells suggests multi-functionality[41,42]. Fain *et al*[43] were the first to demonstrate the predominant expression of omentin mRNA in human epicardial fat, suggesting that omentin might influence coronary atherogenesis like other periadventitial epicardial adipokines. Some researchers reported that omentin might modulate vascular function through direct action on endothelial cells[44,45]. The vasodilating effect of omentin on isolated rat aorta, mediated by endothelium-derived nitric oxide, was first examined by Yamawaki *et al*[45]. Treatment of human endothelial cells with omentin prevented TNF-α-induced cyclooxygenase-2 expression by inhibiting c-Jun N-terminal kinase signaling, suggesting an anti-inflammatory function of omentin on endothelial cells[44]. Recently, several *in vivo* studies that might explain the mechanism underlying the connection between circulating omentin-1 and the atherosclerotic process have been published. In human endothelial cells, omentin significantly decreased C-reactive protein and TNF-α-induced NF-κB[46]. Xie *et al*[47] reported that adenovirus-mediated overexpression of omentin-1 attenuated arterial calcification in OPG$^{-/-}$ mice, suggesting that increasing concentrations of omentin-1 might be beneficial by protecting arteries. In an *in vitro* study, treatment of calcifying vascular smooth muscle cells (CVSMs) with omentin inhibited osteoblastic differentiation of CVSMCs *via* the phosphatidylinositol 3-kinase/Akt signaling pathway[48]. Very recently, Maruyama *et al*[49] reported that systemic delivery of an adenoviral vector expressing omentin enhanced blood flow recovery and capillary density in ischemic limbs of wild type mice. Taken together, these *in vitro* data suggest the possibility that lower omentin levels contribute to the development of cardiovascular disease from initiating early endothelial dysfunction to arterial calcification.

There have been many clinical studies examining the

significance of correlations of circulating omentin-1 levels with brachial artery vascular reactivity, carotid intima media thickness, and coronary heart disease[50-53]. Moreno-Navarrete *et al*[50] demonstrated that the concentration of circulating omentin-1 contributes independently to endothelial dysfunction, even after controlling for adiposity, age, and inflammation in subjects with impaired glucose tolerance. In that study, vascular reactivity was measured using high-resolution ultrasound imaging of the brachial artery. Subsequently, two reports on the negative correlation of serum omentin-1 with carotid IMT have suggested cardioprotective and anti-atherosclerotic roles for omentin-1[52,53]. Liu *et al*[53] demonstrated that the serum omentin-1 level was independently correlated with carotid IMT in metabolic syndrome patients, and Shibata *et al*[52] showed similar results in apparently healthy men. Recently, El-Mesallamy *et al*[54] examined the level of circulating omentin-1 in an Egyptian population with type 2 diabetes, with or without ischemic heart disease. Although they did not detect clear differences in serum omentin-1 levels between patients with type 2 diabetes with or without ischemic heart disease, multiple regression analysis showed that the IL-6 level was an independent risk factor influencing serum omentin-1 level. This suggests that omentin-1 is regulated by inflammation. Therefore, omentin is regarded as a novel link between insulin resistance, inflammation, and cardiovascular disease, suggesting its possibility as a novel therapeutic target for the cardiovascular diseases.

CHEMERIN

Chemerin was identified as a chemoattractant that promotes the recruitment of immature dendritic cells and macrophages to lymphoid organs and sites of tissue injury[55]. Goralski *et al*[56] first reported a high level of chemerin expression in mouse and human adipocytes. They also reported that loss of chemerin expression almost completely abrogated adipogenesis in 3T3-L1 cells, and modified the expression of genes important in glucose and lipid metabolism, such as GLUT4, leptin, and adiponectin[56]. After that, Ernst *et al*[57] reported that exogenous administration of chemerin exacerbated glucose intolerance, lowered serum insulin levels, and decreased tissue glucose uptake in a mouse model of obesity and diabetes. Growing evidence from human data supports a linkage between chemerin, obesity, and metabolic syndrome. A study of a Mexican-American population showed that circulating chemerin levels were significantly higher in obese subjects compared with non-obese controls. Plasma levels of chemerin were correlated positively with body mass index (BMI), fasting glucose, fasting insulin, and triglycerides levels, and negatively correlated with high-density lipoprotein (HDL)-cholesterol level[58]. Bozaoglu *et al*[58,59] demonstrated that serum chemerin levels were closely correlated with BMI, fasting serum insulin, triglycerides, and HDL-cholesterol in non-diabetic subjects. Sell *et al*[60] reported that in patients who had undergone bariatric surgery for weight reduction, the serum chemerin levels were significantly reduced after surgery, indicating that chemerin might mediate the metabolic alterations in obesity.

Although chemerin is a well-known secreted protein with an established role in immune function, recent experimental data indicate that chemerin might provide a link between obesity and chronic inflammation[61]. Recently, Sell *et al*[62] reported that chemerin activated the NF-κB pathway and impaired glucose uptake in primary human skeletal muscle cells. Moreover, TNF-α treatment of 3T3-L1 adipocytes increased bioactive chemerin levels, suggesting that inflammatory cytokines contribute to the up-regulation of chemerin in obesity[63]. Thus, adipocyte-derived chemerin might be involved in the pathogenesis of obesity-related inflammatory disorders, including atherosclerosis. Although Becker *et al*[64] showed that the expression of chemerin did not significantly alter the extent of atherosclerosis in low-density lipoprotein cholesterol receptor knockout mice, they hypothesized that chemerin might affect early atherosclerotic plaque development and morphology rather than the extent of the atherosclerotic lesion area. Hart *et al*[65] showed that chemerin rapidly stimulated the adhesion of macrophages to the extracellular matrix protein, fibronectin, and to the adhesion molecule, vascular cell adhesion molecule-1, suggesting that chemerin might promote the progression of atherosclerosis. Furthermore, Kaur *et al*[66] demonstrated the novel presence of a G-protein coupled chemerin receptor 1 in human endothelial cells and its significant up-regulation by pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6). Thus, the altered expression of chemerin and its receptors during an inflammatory process might cause dysregulated angiogenesis, leading to the development of cardiovascular disease.

However, there have been very few clinical studies that examined the influence of circulating chemerin on the atherosclerotic process. Lehrke *et al*[67] showed that circulating chemerin was positively correlated with the atherosclerotic plaque burden, as assessed by multi-slice computed tomography angiography, but that the association was lost after adjusting for established cardiovascular risk factors. Very recently, we showed that the circulating chemerin level was an independent risk factor for arterial stiffness even after adjusting other cardiovascular risk factors[68].

CONCLUSION

Various adipokines have been reported to directly modulate the atherogenic environment of the vessel wall by regulating the function of endothelial, arterial smooth muscle, and macrophage cells. Therefore, the identification of a novel adipokine that regulates the atherosclerotic process might provide new opportunities for developing more effective approaches for preventing cardiovascular disease.

REFERENCES

1 **Scherer PE**. Adipose tissue: from lipid storage compartment

to endocrine organ. *Diabetes* 2006; **55**: 1537-1545 [PMID: 16731815 DOI: 10.2337/db06-0263]
2 **Ntaios G**, Gatselis NK, Makaritsis K, Dalekos GN. Adipokines as mediators of endothelial function and atherosclerosis. *Atherosclerosis* 2013; **227**: 216-221 [PMID: 23332774 DOI: 10.1016/j.atherosclerosis.2012.12.029]
3 **Maeda K**, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara K. cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant Gene transcript 1). *Biochem Biophys Res Commun* 1996; **221**: 286-289 [PMID: 8619847 DOI: 10.1006/bbrc.1996.0587]
4 **Kadowaki T**, Yamauchi T. Adiponectin and adiponectin receptors. *Endocr Rev* 2005; **26**: 439-451 [PMID: 15897298 DOI: 10.1210/er.2005-0005]
5 **Stefan N**, Stumvoll M. Adiponectin--its role in metabolism and beyond. *Horm Metab Res* 2002; **34**: 469-474 [PMID: 12384822 DOI: 10.1055/s-2002-34785]
6 **Yamauchi T**, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, Sugiyama T, Miyagishi M, Hara K, Tsunoda M, Murakami K, Ohteki T, Uchida S, Takekawa S, Waki H, Tsuno NH, Shibata Y, Terauchi Y, Froguel P, Tobe K, Koyasu S, Taira K, Kitamura T, Shimizu T, Nagai R, Kadowaki T. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature* 2003; **423**: 762-769 [PMID: 12802337 DOI: 10.1038/nature01705]
7 **Matsuzawa Y**. Adiponectin: Identification, physiology and clinical relevance in metabolic and vascular disease. *Atheroscler Suppl* 2005; **6**: 7-14 [PMID: 15823491 DOI: 10.1016/j.atherosclerosissup.2005.02.003]
8 **Ouchi N**, Shibata R, Walsh K. Cardioprotection by adiponectin. *Trends Cardiovasc Med* 2006; **16**: 141-146 [PMID: 16781946 DOI: 10.1016/j.tcm.2006.03.001]
9 **Choi KM**, Lee J, Lee KW, Seo JA, Oh JH, Kim SG, Kim NH, Choi DS, Baik SH. Serum adiponectin concentrations predict the developments of type 2 diabetes and the metabolic syndrome in elderly Koreans. *Clin Endocrinol* (Oxf) 2004; **61**: 75-80 [PMID: 15212647 DOI: 10.1111/j.1365-2265.2004.02063.x]
10 **Pischon T**, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB. Plasma adiponectin levels and risk of myocardial infarction in men. *JAMA* 2004; **291**: 1730-1737 [PMID: 15082700 DOI: 10.1001/jama.291.14.1730]
11 **Ouchi N**, Kihara S, Arita Y, Okamoto Y, Maeda K, Kuriyama H, Hotta K, Nishida M, Takahashi M, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Funahashi T, Matsuzawa Y. Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway. *Circulation* 2000; **102**: 1296-1301 [PMID: 10982546]
12 **Choi HY**, Kim S, Yang SJ, Yoo HJ, Seo JA, Kim SG, Kim NH, Baik SH, Choi DS, Choi KM. Association of adiponectin, resistin, and vascular inflammation: analysis with 18F-fluorodeoxyglucose positron emission tomography. *Arterioscler Thromb Vasc Biol* 2011; **31**: 944-949 [PMID: 21212400 DOI: 10.1161/ATVBAHA.110.220673]
13 **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]
14 **Filková M**, Haluzík M, Gay S, Senolt L. The role of resistin as a regulator of inflammation: Implications for various human pathologies. *Clin Immunol* 2009; **133**: 157-170 [PMID: 19740705 DOI: 10.1016/j.clim.2009.07.013]
15 **Reilly MP**, Lehrke M, Wolfe ML, Rohatgi A, Lazar MA, Rader DJ. Resistin is an inflammatory marker of atherosclerosis in humans. *Circulation* 2005; **111**: 932-939 [PMID: 15710760 DOI: 10.1161/01.CIR.0000155620.10387.43]
16 **Kawanami D**, Maemura K, Takeda N, Harada T, Nojiri T, Imai Y, Manabe I, Utsunomiya K, Nagai R. Direct reciprocal effects of resistin and adiponectin on vascular endothelial cells: a new insight into adipocytokine-endothelial cell interactions. *Biochem Biophys Res Commun* 2004; **314**: 415-419 [PMID: 14733921]
17 **Lee TS**, Lin CY, Tsai JY, Wu YL, Su KH, Lu KY, Hsiao SH, Pan CC, Kou YR, Hsu YP, Ho LT. Resistin increases lipid accumulation by affecting class A scavenger receptor, CD36 and ATP-binding cassette transporter-A1 in macrophages. *Life Sci* 2009; **84**: 97-104 [PMID: 19041881 DOI: 10.1016/j.lfs.2008.11.004]
18 **Piestrzeniewicz K**, Łuczak K, Komorowski J, Maciejewski M, Jankiewicz Wika J, Goch JH. Resistin increases with obesity and atherosclerotic risk factors in patients with myocardial infarction. *Metabolism* 2008; **57**: 488-493 [PMID: 18328349 DOI: 10.1016/j.metabol.2007.11.009]
19 **Qi Q**, Wang J, Li H, Yu Z, Ye X, Hu FB, Franco OH, Pan A, Liu Y, Lin X. Associations of resistin with inflammatory and fibrinolytic markers, insulin resistance, and metabolic syndrome in middle-aged and older Chinese. *Eur J Endocrinol* 2008; **159**: 585-593 [PMID: 18753313 DOI: 10.1530/EJE-08-0427]
20 **Weikert C**, Westphal S, Berger K, Dierkes J, Möhlig M, Spranger J, Rimm EB, Willich SN, Boeing H, Pischon T. Plasma resistin levels and risk of myocardial infarction and ischemic stroke. *J Clin Endocrinol Metab* 2008; **93**: 2647-2653 [PMID: 18460562 DOI: 10.1210/jc.2007-2735]
21 **Lee SH**, Ha JW, Kim JS, Choi EY, Park S, Kang SM, Choi D, Jang Y, Chung N. Plasma adiponectin and resistin levels as predictors of mortality in patients with acute myocardial infarction: data from infarction prognosis study registry. *Coron Artery Dis* 2009; **20**: 33-39 [PMID: 18997620 DOI: 10.1097/MCA.0b013e328318ecb0]
22 **Makowski L**, Hotamisligil GS. Fatty acid binding proteins--the evolutionary crossroads of inflammatory and metabolic responses. *J Nutr* 2004; **134**: 2464S-2468S [PMID: 15333743]
23 **Xu A**, Wang Y, Xu JY, Stejskal D, Tam S, Zhang J, Wat NM, Wong WK, Lam KS. Adipocyte fatty acid-binding protein is a plasma biomarker closely associated with obesity and metabolic syndrome. *Clin Chem* 2006; **52**: 405-413 [PMID: 16423904 DOI: 10.1373/clinchem.2005.062463]
24 **Uysal KT**, Scheja L, Wiesbrock SM, Bonner-Weir S, Hotamisligil GS. Improved glucose and lipid metabolism in genetically obese mice lacking aP2. *Endocrinology* 2000; **141**: 3388-3396 [PMID: 10965911]
25 **Tso AW**, Xu A, Sham PC, Wat NM, Wang Y, Fong CH, Cheung BM, Janus ED, Lam KS. Serum adipocyte fatty acid binding protein as a new biomarker predicting the development of type 2 diabetes: a 10-year prospective study in a Chinese cohort. *Diabetes Care* 2007; **30**: 2667-2672 [PMID: 17620449 DOI: 10.2337/dc07-0413]
26 **Xu A**, Tso AW, Cheung BM, Wang Y, Wat NM, Fong CH, Yeung DC, Janus ED, Sham PC, Lam KS. Circulating adipocyte-fatty acid binding protein levels predict the development of the metabolic syndrome: a 5-year prospective study. *Circulation* 2007; **115**: 1537-1543 [PMID: 17389279 DOI: 10.1161/CIRCULATIONAHA.106.647503]
27 **Boord JB**, Maeda K, Makowski L, Babaev VR, Fazio S, Linton MF, Hotamisligil GS. Combined adipocyte-macrophage fatty acid-binding protein deficiency improves metabolism, atherosclerosis, and survival in apolipoprotein E-deficient mice. *Circulation* 2004; **110**: 1492-1498 [PMID: 15353487 DOI: 10.1161/01.CIR.0000141735.13202.B6]
28 **Fu Y**, Luo N, Lopes-Virella MF, Garvey WT. The adipocyte lipid binding protein (ALBP/aP2) gene facilitates foam cell formation in human THP-1 macrophages. *Atherosclerosis* 2002; **165**: 259-269 [PMID: 12417276]
29 **Makowski L**, Brittingham KC, Reynolds JM, Suttles J, Hotamisligil GS. The fatty acid-binding protein, aP2, coordinates macrophage cholesterol trafficking and inflammatory activity. Macrophage expression of aP2 impacts peroxisome proliferator-activated receptor gamma and IkappaB kinase activities. *J Biol Chem* 2005; **280**: 12888-12895 [PMID: 15684432 DOI: 10.1074/jbc.M413788200]

30 **Makowski L**, Boord JB, Maeda K, Babaev VR, Uysal KT, Morgan MA, Parker RA, Suttles J, Fazio S, Hotamisligil GS, Linton MF. Lack of macrophage fatty-acid-binding protein aP2 protects mice deficient in apolipoprotein E against atherosclerosis. *Nat Med* 2001; **7**: 699-705 [PMID: 11385507 DOI: 10.1038/89076]

31 **Furuhashi M**, Tuncman G, Görgün CZ, Makowski L, Atsumi G, Vaillancourt E, Kono K, Babaev VR, Fazio S, Linton MF, Sulsky R, Robl JA, Parker RA, Hotamisligil GS. Treatment of diabetes and atherosclerosis by inhibiting fatty-acid-binding protein aP2. *Nature* 2007; **447**: 959-965 [PMID: 17554340 DOI: 10.1038/nature05844]

32 **Rhee EJ**, Lee WY, Park CY, Oh KW, Kim BJ, Sung KC, Kim BS. The association of serum adipocyte fatty acid-binding protein with coronary artery disease in Korean adults. *Eur J Endocrinol* 2009; **160**: 165-172 [PMID: 19001529 DOI: 10.1530/EJE-08-0665]

33 **Yeung DC**, Xu A, Cheung CW, Wat NM, Yau MH, Fong CH, Chau MT, Lam KS. Serum adipocyte fatty acid-binding protein levels were independently associated with carotid atherosclerosis. *Arterioscler Thromb Vasc Biol* 2007; **27**: 1796-1802 [PMID: 17510463 DOI: 10.1161/ATVBAHA.107.146274]

34 **Miyoshi T**, Onoue G, Hirohata A, Hirohata S, Usui S, Hina K, Kawamura H, Doi M, Kusano KF, Kusachi S, Ninomiya Y. Serum adipocyte fatty acid-binding protein is independently associated with coronary atherosclerotic burden measured by intravascular ultrasound. *Atherosclerosis* 2010; **211**: 164-169 [DOI: 10.1016/j.atherosclerosis.2010.01.032]

35 **Yoo HJ**, Kim S, Park MS, Choi HY, Yang SJ, Seo JA, Kim SG, Kim NH, Baik SH, Choi DS, Choi KM. Serum adipocyte fatty acid-binding protein is associated independently with vascular inflammation: analysis with (18)F-fluorodeoxyglucose positron emission tomography. *J Clin Endocrinol Metab* 2011; **96**: E488-E492 [PMID: 21159847 DOI: 10.1210/jc.2010-1473]

36 **Yang R**, Xu A, Pray J, Hu H, Jadhao S, Hansen B, Shuldiner A, Mc-Lenithan J, Gong D. Cloning of omentin, a new adipocytokine from omental fat tissue in humans. *Diabetes* 2003; Suppl1: A1

37 **Yang RZ**, Lee MJ, Hu H, Pray J, Wu HB, Hansen BC, Shuldiner AR, Fried SK, McLenithan JC, Gong DW. Identification of omentin as a novel depot-specific adipokine in human adipose tissue: possible role in modulating insulin action. *Am J Physiol Endocrinol Metab* 2006; **290**: E1253-E1261 [PMID: 16531507 DOI: 10.1152/ajpendo.00572.2004]

38 **Pan HY**, Guo L, Li Q. Changes of serum omentin-1 levels in normal subjects and in patients with impaired glucose regulation and with newly diagnosed and untreated type 2 diabetes. *Diabetes Res Clin Pract* 2010; **88**: 29-33 [PMID: 20129687 DOI: 10.1016/j.diabres.2010.01.013]

39 **Tan BK**, Adya R, Farhatullah S, Lewandowski KC, O'Hare P, Lehnert H, Randeva 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]

40 **de Souza Batista CM**, Yang RZ, Lee MJ, Glynn NM, Yu DZ, Pray J, Ndubuizu 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]

41 **Tsuji S**, Uehori J, Matsumoto M, Suzuki Y, Matsuhisa A, Toyoshima K, Seya T. Human intelectin is a novel soluble lectin that recognizes galactofuranose in carbohydrate chains of bacterial cell wall. *J Biol Chem* 2001; **276**: 23456-23463 [PMID: 11313366 DOI: 10.1074/jbc.M103162200]

42 **Suzuki YA**, Shin K, Lönnerdal B. Molecular cloning and functional expression of a human intestinal lactoferrin receptor. *Biochemistry* 2001; **40**: 15771-15779 [PMID: 11747454 DOI: 10.1021/bi0155899]

43 **Fain JN**, Sacks HS, Buehrer B, Bahouth SW, Garrett E, Wolf RY, Carter RA, Tichansky DS, Madan AK. Identification of omentin mRNA in human epicardial adipose tissue: comparison to omentin in subcutaneous, internal mammary artery periadventitial and visceral abdominal depots. *Int J Obes* (Lond) 2008; **32**: 810-815 [PMID: 18180782 DOI: 10.1038/sj.ijo.0803790]

44 **Yamawaki H**, Kuramoto J, Kameshima S, Usui T, Okada M, Hara Y. Omentin, a novel adipocytokine inhibits TNF-induced vascular inflammation in human endothelial cells. *Biochem Biophys Res Commun* 2011; **408**: 339-343 [PMID: 21514279 DOI: 10.1016/j.bbrc.2011.04.039]

45 **Yamawaki H**, Tsubaki N, Mukohda M, Okada M, Hara Y. Omentin, a novel adipokine, induces vasodilation in rat isolated blood vessels. *Biochem Biophys Res Commun* 2010; **393**: 668-672 [PMID: 20170632 DOI: 10.1016/j.bbrc.2010.02.053]

46 **Tan BK**, Adya R, Farhatullah S, Chen J, Lehnert H, Randeva 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]

47 **Xie H**, Xie PL, Wu XP, Chen SM, Zhou HD, Yuan LQ, Sheng ZF, Tang SY, Luo XH, Liao EY. Omentin-1 attenuates arterial calcification and bone loss in osteoprotegerin-deficient mice by inhibition of RANKL expression. *Cardiovasc Res* 2011; **92**: 296-306 [DOI: 10.1093/cvr/cvr200]

48 **Duan XY**, Xie PL, Ma YL, Tang SY. Omentin inhibits osteoblastic differentiation of calcifying vascular smooth muscle cells through the PI3K/Akt pathway. *Amino Acids* 2011; **41**: 1223-1231 [PMID: 21082206 DOI: 10.1007/s00726-010-0800-3]

49 **Maruyama S**, Shibata R, Kikuchi R, Izumiya Y, Rokutanda T, Araki S, Kataoka Y, Ohashi K, Daida H, Kihara S, Ogawa H, Murohara T, Ouchi N. Fat-derived factor omentin stimulates endothelial cell function and ischemia-induced revascularization via endothelial nitric oxide synthase-dependent mechanism. *J Biol Chem* 2012; **287**: 408-417 [PMID: 22081609 DOI: 10.1074/jbc.M111.261818]

50 **Moreno-Navarrete JM**, Ortega F, Castro A, Sabater M, Ricart W, Fernández-Real JM. Circulating omentin as a novel biomarker of endothelial dysfunction. *Obesity* (Silver Spring) 2011; **19**: 1552-1559 [PMID: 21293447 DOI: 10.1038/oby.2010.351]

51 **Shibata R**, Ouchi N, Kikuchi R, Takahashi R, Takeshita K, Kataoka Y, Ohashi K, Ikeda N, Kihara S, Murohara T. Circulating omentin is associated with coronary artery disease in men. *Atherosclerosis* 2011; **219**: 811-814 [PMID: 21925659 DOI: 10.1016/j.atherosclerosis.2011.08.017]

52 **Shibata R**, Takahashi R, Kataoka Y, Ohashi K, Ikeda N, Kihara S, Murohara T, Ouchi N. Association of a fat-derived plasma protein omentin with carotid artery intima-media thickness in apparently healthy men. *Hypertens Res* 2011; **34**: 1309-1312 [PMID: 21814208 DOI: 10.1038/hr.2011.130]

53 **Liu R**, Wang X, Bu P. Omentin-1 is associated with carotid atherosclerosis in patients with metabolic syndrome. *Diabetes Res Clin Pract* 2011; **93**: 21-25 [DOI: 10.1016/j.diabres.2011.03.001]

54 **El-Mesallamy HO**, El-Derany MO, Hamdy NM. Serum omentin-1 and chemerin levels are interrelated in patients with Type 2 diabetes mellitus with or without ischaemic heart disease. *Diabet Med* 2011; **28**: 1194-1200 [PMID: 21668495 DOI: 10.1111/j.1464-5491.2011.03353.x]

55 **Wittamer V**, Franssen JD, Vulcano M, Mirjolet JF, Le Poul E, Migeotte I, Brézillon S, Tyldesley R, Blanpain C, Detheux M, Mantovani A, Sozzani S, Vassart G, Parmentier M, Communi D. Specific recruitment of antigen-presenting cells by chemerin, a novel processed ligand from human inflammatory fluids. *J Exp Med* 2003; **198**: 977-985 [PMID: 14530373 DOI: 10.1084/jem.20030382]

56 **Goralski KB**, McCarthy TC, Hanniman EA, Zabel BA, Butcher EC, Parlee SD, Muruganandan S, Sinal CJ. Chemerin, a novel adipokine that regulates adipogenesis and adipocyte metabolism. *J Biol Chem* 2007; **282**: 28175-28188 [PMID:

17635925 DOI: 10.1074/jbc.M700793200]
57 **Ernst MC**, Issa M, Goralski KB, Sinal CJ. Chemerin exacerbates glucose intolerance in mouse models of obesity and diabetes. *Endocrinology* 2010; **151**: 1998-2007 [PMID: 20228173 DOI: 10.1210/en.2009-1098]
58 **Bozaoglu K**, Segal D, Shields KA, Cummings N, Curran JE, Comuzzie AG, Mahaney MC, Rainwater DL, VandeBerg JL, MacCluer JW, Collier G, Blangero J, Walder K, Jowett JB. Chemerin is associated with metabolic syndrome phenotypes in a Mexican-American population. *J Clin Endocrinol Metab* 2009; **94**: 3085-3088 [PMID: 19470637 DOI: 10.1210/jc.2008-1833]
59 **Bozaoglu K**, Bolton K, McMillan J, Zimmet P, Jowett J, Collier G, Walder K, Segal D. Chemerin is a novel adipokine associated with obesity and metabolic syndrome. *Endocrinology* 2007; **148**: 4687-4694 [PMID: 17640997 DOI: 10.1210/en.2007-0175]
60 **Sell H**, Divoux A, Poitou C, Basdevant A, Bouillot JL, Bedossa P, Tordjman J, Eckel J, Clément K. Chemerin correlates with markers for fatty liver in morbidly obese patients and strongly decreases after weight loss induced by bariatric surgery. *J Clin Endocrinol Metab* 2010; **95**: 2892-2896 [PMID: 20375212 DOI: 10.1210/jc.2009-2374]
61 **Ernst MC**, Sinal CJ. Chemerin: at the crossroads of inflammation and obesity. *Trends Endocrinol Metab* 2010; **21**: 660-667 [PMID: 20817486 DOI: 10.1016/j.tem.2010.08.001]
62 **Sell H**, Laurencikiene J, Taube A, Eckardt K, Cramer A, Horrighs A, Arner P, Eckel J. Chemerin is a novel adipocyte-derived factor inducing insulin resistance in primary human skeletal muscle cells. *Diabetes* 2009; **58**: 2731-2740 [PMID: 19720798 DOI: 10.2337/db09-0277]
63 **Parlee SD**, Ernst MC, Muruganandan S, Sinal CJ, Goralski KB. Serum chemerin levels vary with time of day and are modified by obesity and tumor necrosis factor-{alpha}. *Endocrinology* 2010; **151**: 2590-2602 [PMID: 20363880 DOI: 10.1210/en.2009-0794]
64 **Becker M**, Rabe K, Lebherz C, Zugwurst J, Göke B, Parhofer KG, Lehrke M, Broedl UC. Expression of human chemerin induces insulin resistance in the skeletal muscle but does not affect weight, lipid levels, and atherosclerosis in LDL receptor knockout mice on high-fat diet. *Diabetes* 2010; **59**: 2898-2903 [PMID: 20724582 DOI: 10.2337/db10-0362]
65 **Hart R**, Greaves DR. Chemerin contributes to inflammation by promoting macrophage adhesion to VCAM-1 and fibronectin through clustering of VLA-4 and VLA-5. *J Immunol* 2010; **185**: 3728-3739 [PMID: 20720202 DOI: 10.4049/jimmunol.0902154]
66 **Kaur J**, Adya R, Tan BK, Chen J, Randeva HS. Identification of chemerin receptor (ChemR23) in human endothelial cells: chemerin-induced endothelial angiogenesis. *Biochem Biophys Res Commun* 2010; **391**: 1762-1768 [PMID: 20044979 DOI: 10.1016/j.bbrc.2009.12.150]
67 **Lehrke M**, Becker A, Greif M, Stark R, Laubender RP, von Ziegler F, Lebherz C, Tittus J, Reiser M, Becker C, Göke B, Leber AW, Parhofer KG, Broedl UC. Chemerin is associated with markers of inflammation and components of the metabolic syndrome but does not predict coronary atherosclerosis. *Eur J Endocrinol* 2009; **161**: 339-344 [PMID: 19497986 DOI: 10.1530/EJE-09-0380]
68 **Yoo HJ**, Choi HY, Yang SJ, Kim HY, Seo JA, Kim SG, Kim NH, Choi KM, Choi DS, Baik SH. Circulating chemerin level is independently correlated with arterial stiffness. *J Atheroscler Thromb* 2012; **19**: 59-66; discussion 67-68 [PMID: 22104178 DOI: 10.5551/jat.9647]

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REVIEW

Adrenomedullin and diabetes

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Abstract

Adrenomedullin (ADM) is a peptide hormone widely expressed in different tissues, especially in the vasculature. Apart from its vasodilatatory and hypotensive effect, it plays multiple roles in the regulation of hormonal secretion, glucose metabolism and inflammatory response. ADM regulates insulin balance and may participate in the development of diabetes. The plasma level of ADM is increased in people with diabetes, while in healthy individuals the plasma ADM concentration remains low. Plasma ADM levels are further increased in patients with diabetic complications. In type 1 diabetes, plasma ADM level is correlated with renal failure and retinopathy, while in type 2 diabetes its level is linked with a wider range of complications. The elevation of ADM level in diabetes may be due to hyperinsulinemia, oxidative stress and endothelial injury. At the same time, a rise in plasma ADM level can trigger the onset of diabetes. Strategies to reduce ADM level should be explored so as to reduce diabetic complications.

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Key words: Adrenomedullin; Diabetes; Diabetic complications; Hyperglycemia; Therapeutics

Core tip: Adrenomedullin (ADM) is a peptide hormone with vasorelaxing and hypotensive properties. It also plays multiple roles in the regulation of hormonal secretion, glucose metabolism and inflammatory response. A major observation is the elevation of plasma ADM level in diabetes, and is associated with diabetic complications in both type 1 and 2 diabetes. The increase could be resulted from oxidative stress, hyperinsulinemia and endothelial injury. This raises the potential application of ADM as a marker in diabetes, and strategies aimed at reducing ADM level could be explored so as to alleviate diabetic complications.

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INTRODUCTION

Adrenomedullin (ADM) is a peptide recently discovered with multiple functions. Its characteristic actions include vasorelaxing effect and hypotensive properties. Given its widespread expression and production in different organs, ADM can also act as an autocrine, endocrine or paracrine mediator in various biological systems. The prospects of ADM as a potential disease modulator comes from the observation of increased levels in plasma in various disease states. For instance, increased plasma ADM levels were observed in cardiovascular diseases and diabetes[1-3]. However, different from the observations in cardiovascular diseases, the explanation and significance for such an increase is not clear. Since then, research progress has been made in the association between ADM

and diabetes. For instance, ADM plays a role in glucose metabolism and insulin balance[4]. These evidence may provide clue on the involvement of ADM in diabetes.

In this review, we summarized the current knowledge on ADM based on research progress in the recent decade and provided an account on the role of ADM played in the context of diabetes. This would help us understand better on the clinical application of ADM in diabetic patients.

DISCOVERY OF ADRENOMEDULLIN AS A REGULATORY PEPTIDE

ADM was initially discovered by Kitamura in 1993, extracted from pheochromocytoma in humans by monitoring the elevated 3',5' cyclic adenosine monophosphate (cAMP) production in human platelets[5]. It was later found that the peptide had a potent hypotensive and vasorelaxing effects. It forms a ring structure by 52 amino acid residues held by a disulfide bond. Since the peptide was abundantly found in the adrenal medulla, therefore this accounts for the name. The peptide is classified as a member of the calcitonin gene-related peptide (CGRP) superfamily. Although high level of ADM was identified in the adrenal medulla[6], circulating ADM was the most abundant in vascular wall[7].

BIOSYNTHESIS AND DISTRIBUTION

ADM has a very high tissue distribution. Its biosynthesis has been studied by applying radioimmunoassays, and by detecting tissue ADM mRNA[8]. Immunoreactive ADM is detected in cardiovascular, respiratory, renal, endocrine, reproductive, neurological, intestinal and immune system[9,10]. Among these systems the highest ADM concentrations were detected at the adrenal glands. ADM mRNA is also detected in various peripheral tissues[11]. Such wide distributions indicate the multi-facet roles of ADM.

In the cardiovascular system, ADM is synthesized in both atria and ventricles in heart and blood vessels. Within the vasculature, ADM is actively manufactured and secreted by both the endothelial and the vascular smooth muscle cells[7,12]. It is also demonstrated that the vasculature had much higher ADM mRNA expression than the adrenal glands. This was further supported by the finding of a low ADM precursor ratio in the total ADM immunoreactivity in blood vessels[11].

Besides, ADM is synthesized in the lung[13], brain as well as in the pancreatic islets[14,15]. The widespread ADM expression suggests its diverse role in the regulations of cell functions. Since ADM is mainly produced by vascular endothelial and the smooth muscle cells, its regulatory function of vascular tone has become a major target for investigation.

ADM production is controlled by various humoral factors and physical factors. Inflammatory cytokines such as tumor necrosis factor (TNF)-α, TNF-β, interleukin (IL)-1α and IL-1β all are known to stimulate ADM production and secretion[16]. While mechanical factors like sheer stress and hypoxia are involved in the up-regulation of vascular ADM mRNA expression[17].

In healthy individuals, circulating plasma ADM level is as low as in the picomolar range, similar to the atrial natriuretic peptide, and its level changes in order to compensate for the vasoconstrictive effects. It is reported that in various pathological conditions, the increase in plasma ADM level correlates with severity of disease states. For instance, elevated plasma ADM level has been associated with heart failure, hypertension, artherosclerosis and diabetes mellitus[18].

RECEPTOR SIGNALING

Specific binding sites for ADM were identified in many different places in rat and in human models[19,20]. In humans, the binding sites are most abundant in the microvascular endothelium[20]. The biological actions of ADM are exerted mainly through CGRP receptors and the specific ADM receptors, which share a common molecular component of a G-protein coupled receptor called calcitonin receptor-like receptor (CRLR)[21]. The specificity of CRLR depends on different subtypes of another associated proteins, namely the receptor-activity-modifying proteins (RAMP1, 2 and 3)[22]. Co-expression of CRLR with different subtypes of RAMPs will form different ADM receptors. The specificity brought about by the RAMPs involves glycosylation and transport of the receptor-RAMP complex.

PHYSIOLOGICAL EFFECTS

ADM can act as both a hormone and a cytokine to regulate the regional blood flow, vascular tone, leukocyte migration and differentiation, electrolyte balance, cardiac function, glucose uptake and hormone secretion[18]. It plays an important role in cardiovascular system[23]. ADM imposes a potent vasodilatory effect in humans and increases blood flow to various organs[24,25]. For instance, increased ADM expression could enhance hepatic and renal circulation[26]. In systemic circulation, vasodilation could be resulted from either endothelium-dependent[27], or endothelium-independent mechanisms[28], through ADM and CGRP receptors. In addition, the endothelium-derived vasodilation could be mediated by cAMP and nitric oxide[29,30].

Previous studies have identified the role of ADM in inflammation and immunity. ADM possesses anti-microbial properties against bacteria[31]. *In vitro* and *in vivo* study has demonstrated that ADM secretion and expression are up-regulated upon pathogenic exposure[32]. ADM expression also increases during local inflammation and sepsis[33] In particular, ADM levels in lung, heart and vasculature[34], liver and kidney[26], all increase upon endotoxin administration[35]. Macrophages could also augment ADM expression in inflammation[33].

The role of ADM in the inflammatory process var-

ies after the onset of inflammation. ADM can activate and modulate cytokine production, while it can also inhibit overproduction of pro-inflammatory cytokines[36]. It plays a crucial role in initiating inflammatory response by stimulating the release of migratory inhibitory factor and IL-1β, while activate anti-inflammatory response by suppressing TNF-α production and up-regulating IL-6 production, as the latter is anti-inflammatory and inhibit lipopolysaccharide-induced TNF-α production[37-39]. Such co-ordinated functions of ADM suggest that it is associated with injury, infection and inflammation. Apart from inflammation, ADM expression in immune cells serves diverse functions. ADM can be detected in macrophages in the atherosclerotic plaques[40], where it may play a role in reducing inflammation and thereby exerting an anti-atherosclerotic effect.

While circulating ADM in plasma contributes to a large part of its physiological functions, ADM also serves as a local regulator of cellular functions. The paracrine effect of ADM can be demonstrated in the kidney, as it has been shown that ADM is histochemically localized in renal tubules, and recently mesangium was suggested to be one source of ADM in the kidney[41]. The local ADM modulates mesangial proliferation and is regulated by different growth factors and cytokines. This suggests that regulation of renal function by ADM may operate in an autocrine/paracrine manner. Another example of the localized effect of ADM is in the vascular smooth muscle cells, where its biosynthesis is regulated through a feedback loop. In one study, stimulation of ADM mRNA levels was observed together with a decrease in the immunoreactive ADM peptide secretion resulted from glycolytic inhibition[42]. As ADM could inhibit vascular smooth muscle cell migration and proliferation in response to growth factors[43], a decreased ADM secretion might stimulate its migration and growth locally, and lead to remodeling upon vascular injuries.

ADRENOMEDULLIN AND PANCREATOLOGY

ADM is deeply involved in pancreatic endocrinology, mainly in insulin secretion[44]. It is known that ADM, CRLR and RAMPs are both expressed in the islets of the pancreas[45]. Previous findings demonstrated that exogenous ADM added to freshly isolated rat islets led to a dose-dependent inhibition of insulin secretion by 78% at 1 μmol/L ADM, and was accompanied by cAMP elevation[3]. Oral glucose tolerance tests have illustrated injection of ADM lowered insulin levels in blood by 2 folds 20 min after glucose administration, accompanied by an increase in circulating glucose[4]. This supports a role of ADM in insulin regulation in pancreas, and implies that ADM is associated with hyperglycemia[46].

Another function of ADM is inhibiting amylase secretion in pancreatic acini[47]. As ADM receptors were not identified in the acini, this suggest that such inhibition is mediated through other receptors[45].

ADRENOMEDULLIN AND DIABETES

As suggested above, ADM inhibits insulin release after an oral glucose load. Therefore, it can be expected that ADM contributes to diabetes and even leads to the development of diabetic complications[48].

Diabetes is characterized by hyperglycemia. It is resulted from dysregulation of insulin secretion or peripherial resistance. Diabetes mellitus causes retinopathy, neuropathy, nephropathy, and atherosclerosis. These complications are the results of prolonged hyperglycemia, altered metabolic pathways and non-enzymatic glycation of proteins[49].

There have been advances in the understanding of the relationship between ADM and diabetes. Plasma ADM level is elevated in patients with poorly controlled diabetes than in normal subjects, which suggests a direct effect of glucose on ADM release[1]. The effect of hyperglycemia on ADM expression is mediated through protein kinase C in vascular smooth muscle cells[50]. The observation that ADM expression in aorta, but not in adrenal gland, was raised in diabetic rats (plasma glucose = 567 ± 167 mg/dL) compared to control (plasma glucose = 94 ± 10 mg/dL), suggests that ADM expression in the vasculature could be the source of plasma ADM in diabetic patients[50]. In the streptozotocin-diabetic rat, there were increases in ADM synthesis in the ventricles and possible ADM secretion in the ventricles, atria and the thoracic aorta[51]. On the other hand, ADM may reduce the levels of inflammatory cytokines and endothelin in the adipose tissue and the skeletal muscle and hence increase glucose uptake[37].

However, another study examining the relationship between plasma ADM level and clinical parameters of diabetes demonstrated contradictory results. It showed no significant difference in plasma ADM level between diabetic patients without nephropathy and normal individuals, despite a significant higher level of HbA1c and plasma glucose in patients with diabetes[52]. Therefore, patients with renal impairment should be excluded when examining the relationship between plasma ADM level and blood glucose level, since patients with renal impairment might demonstrate an increase in the plasma ADM levels. Despite the direct effect of circulating glucose on plasma ADM level has not been well established, a positive association between plasma ADM level and the mean blood pressure has been demonstrated in the same study. Given the high plasma ADM levels in various disorders[53], the elevated ADM levels in diabetes might suggest that it has a protective role. Earlier research also showed an elevated plasma ADM level in patients with hypertension and chronic renal failure, particularly a 3-fold elevation in plasma ADM level associated with more severe renal failure. The elevation in ADM may help to prevent blood pressure increase and body fluid retention[54], and represent a compensatory mechanism for diabetic

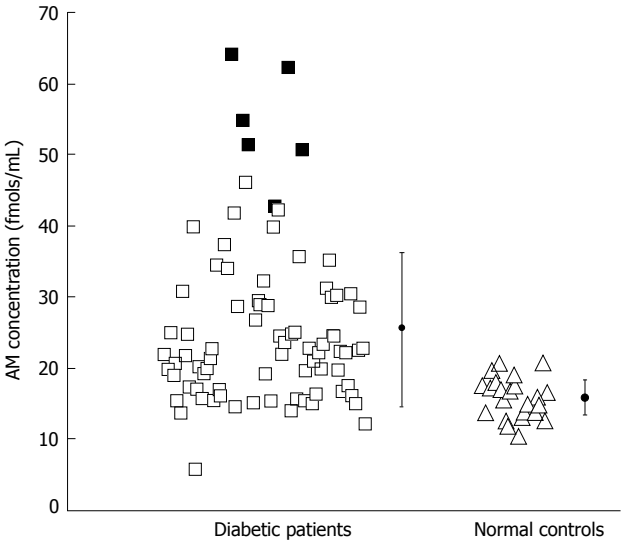

Figure 1 Adrenomedullin concentrations in blood serum from type 2 diabetic patients (in squares) and normal controls (in triangles), shaded squares are outliers. Reprinted from [63].

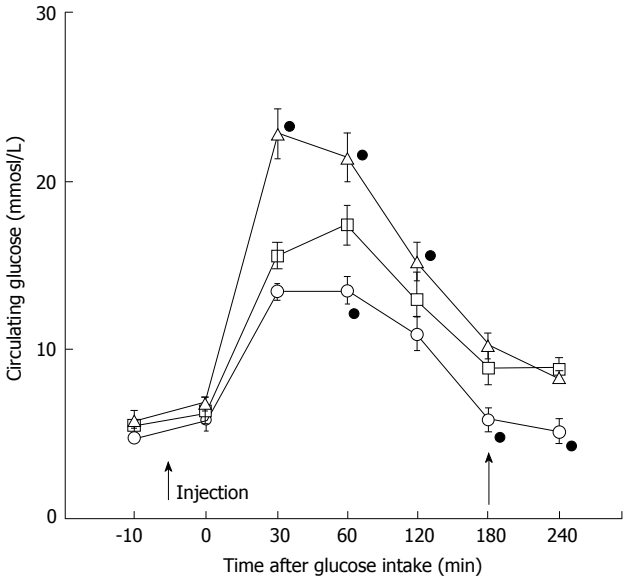

Figure 2 Glucose tolerance test in obese diabetic SHR/N-cp rats after injection of saline (squares), adrenomedullin (triangles) and anti-adrenomedullin monoclonal antibody MoAb-G6 (circles). Black spots indicates point with significant difference compared with saline controls. Modified and reprinted from [63].

complications.

ADM AND TYPE 1 DIABETES

One characteristic of type 1 diabetes is the destruction of β-cells in the islets of Langerhans which produces insulin. Previously there was a report investigating the association of ADM and type 1 diabetes. ADM and cAMP levels were compared between type 1 diabetes patients with various complications and healthy individuals[55]. According to the data, increased plasma ADM level was identified only in patients having renal insufficiency, while patients with other complications had normal ADM level. A significant inverse correlation was also found between ADM levels and the creatinine clearance by multiple regression analysis. This suggested that when the kidney function was impaired, clearance of ADM was possibly decreased and resulted in an increase in the plasma level. Such hypothesis deserves further confirmation because most of the circulating ADM was shown to be cleared in the lungs instead of the kidneys[56]. In the same analysis, the relationship between the plasma ADM and the disease duration suggested the change in ADM level is resulted from the endothelial dysfunction.

Despite the uncertainty of the origin of plasma ADM, a recent study postulated that the selective dilation of glomerular capillaries in type 1 diabetes was attributed to the up-regulation of ADM and RAMP2 expression in the afferent arterioles and glomeruli, through the induced release of nitric oxide[57]. This may provide a hint that locally produced ADM can elicit vasodilatation action by paracrine control, independent of any changes in plasma ADM levels. ADM is also involved in the pathogenesis of retinopathy[58]. Since ADM is produced in the vasculature, endothelial activation caused by vessel damage may explain the increase in plasma ADM level. Another possibility is that ADM acts as a factor for survival of the endothelial cells[59], so plasma level of ADM increases upon endothelial injury. A significant positive association between ADM and cAMP in diabetic patients further supported the hypothesis that ADM plays a counter-regulatory role to prevent excessive vasoconstriction and vessel damage, and promotes natriuresis[54,60,61].

All these findings suggested an increase in plasma ADM level is the consequence rather than the cause of type 1 diabetes, since there are insufficient findings to demonstrate the direct link between ADM and the disease states. This can be further supported by the comparison of hypoglycemic- and hyperglycemic-patients in the same study in which no difference in the plasma ADM level was found.

ADM AND TYPE 2 DIABETES

Several studies have been carried out in an attempt to explain the rise in plasma ADM level and its implications in diabetic complications. One study showed that plasma ADM level was elevated in type 2 diabetes but did not correlate with glucose level in circulation[62]. Instead, increased ADM level was correlated with various diabetic complications, and the severity of diabetic nephropathy and retinopathy. Other parameters like serum creatinine level, systolic blood pressure, and urinary protein excretion were found to be related to ADM levels as well. ADM levels might therefore be related to the development of microangiopathy.

Another study examined a group of patients with a common feature of hyperglycemia development. The group had recent onset of diabetes induced by a drug treatment[63]. Results showed that the group can be characterized by a subset of patients with extremely high

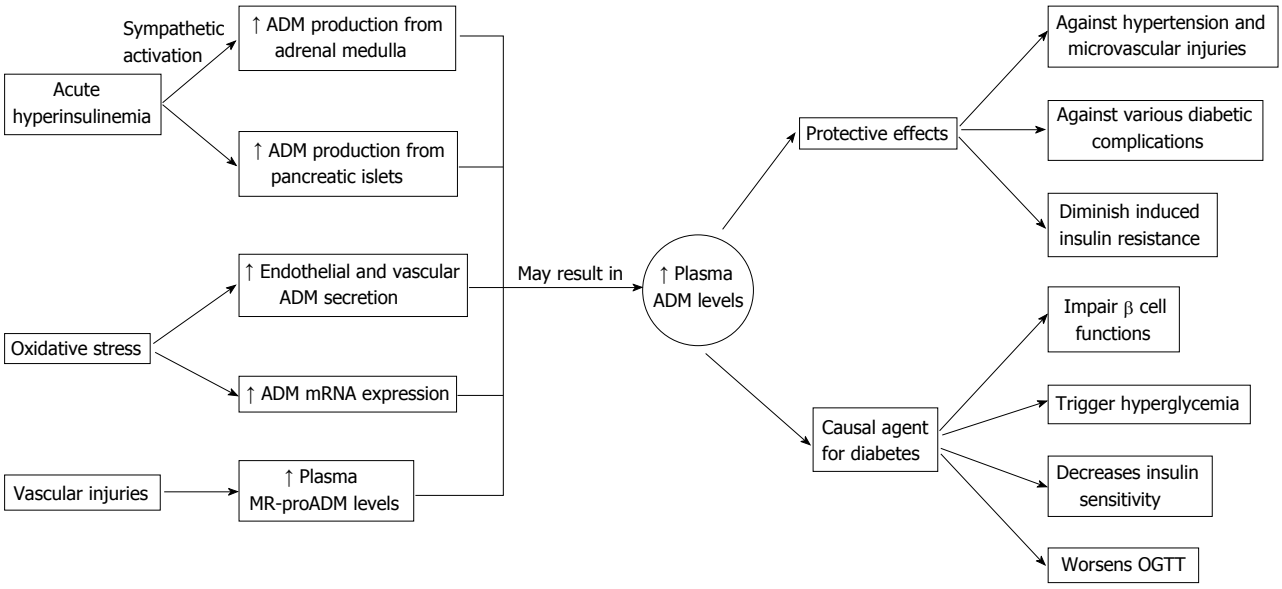

Figure 3 Effects of different disease stresses on subsequent adrenomedullin production and plasma adrenomedullin levels, and the possible roles of increased adrenomedullin levels in type 2 diabetes. ADM: Adrenomedullin.

ADM levels (Figure 1). Even though the source of such excessive ADM is unknown, the results suggested that hyperglycemic patients are characterized by higher circulating ADM levels. In the same studies, the influence of ADM in blood glucose modulation was studied using an obese SHR rat model mimicking human type 2 diabetes. Synthetic ADM, blocking monoclonal antibody against ADM or saline were injected into the animals, and then glucose tolerance tests were carried out. In support to a previous study[4], ADM injection increased blood glucose level more significantly in diabetic rats, while application of antibody effectively reduced blood glucose level to even lower than saline control and improved postprandial recovery in diabetic rats (Figure 2). All these data raise the possibility that ADM is a causative factor in type 2 diabetes and has a negative impact on glycemic control.

To further explore the role of ADM incausing type 2 diabetes, the effect of ADM on insulin secretion has to be considered. There are studies addressing the association of ADM with insulin balance. There is a positive association between insulin resistance and plasma midregion pro-adrenomedullin levels[64]. The link between acute hyperinsulinemia and ADM has been proposed, in which plasma ADM levels increased in acute hyperinsulinemia[65]. There was a concomitant increase in plasma ADM levels with increasing insulin production, and a significant positive correlation between serum insulin levels and plasma ADM was seen in type 2 diabetic patients. The authors speculated that the increased insulin-stimulated ADM production from the pancreatic islets compensated for the diminished vasodilatory effect of insulin, hence this protects against arterial hypertension.

In the recent decade the effect of oxidative stress on ADM expression has been suggested. One study evaluated such relationship by measuring plasma levels of 8-epi-prostaglandin F2α (8-epi-PGF2α, a marker of oxidative stress) and ADM in normal and hypertensive subjects[66]. Both plasma levels were elevated in the hypertensive group ($P < 0.05$ for 8-epi-PGF2α and $P < 0.02$ for ADM respectively), and the data showed that 8-epi-PGF2α was associated with ADM in hypertensive patients with type 2 diabetes ($r = 0.696$, $P < 0.01$). It is known that oxidative stress could stimulate ADM mRNA expression and secretion from endothelial and vascular smooth muscle cells[67]. Sustained ADM deficiency increased oxidative stress and led to insulin resistance *via* impaired insulin signaling, which is supported by an angiotensin (Ang)-Ⅱ treated mouse model[68]. Ang-Ⅱ could induce oxidative stress and hypertensive conditions, and it was shown that Ang-Ⅱ reduced insulin sensitivity in ADM-knockout heterozygous mice more than wild type mice. This suggests that endogenous ADM may act against insulin resistance induced by oxidative stress and offer protection from organ damage through its anti-oxidant action.

The interactions between ADM and diabetic complications are dynamic and complex. While conflicting arguments have been put forward to the link between poor metabolic control and increased ADM levels[64], it is generally accepted that plasma ADM levels are positively linked to oxidative stress[66], acute hyperinsulinemia[65], and other risk factors causing endothelial injury (Figure 3). This leaves much ground for further research about the causes and significance for the plasma ADM level increase.

CONCLUSION

There are two main questions that have to be answered in order to establish a link between ADM and diabetes: Firstly, what are the causes for the increase in plasma ADM levels in diabetic patients, and what are the sources for the elevated circulating ADM? What kind of

stress or stimulation are involved? Secondly, what is the implication for the elevated level? Would it further worsen the glycemic condition and result in various diabetic complications?

Based on the above questions, numerous studies have been commenced. Research has demonstrated the association between diabetic complications and the increase in plasma ADM level. Plasma ADM levels were mainly associated with renal failure and retinopathy in type 1 diabetes. However, the correlation with hyperglycemia is still not clear and requires further investigation.

On the other hand, plasma ADM levels in type 2 diabetes patients are linked to a wider range of complications. The rise may be attributed to acute hyperinsulinemia, oxidative stress and endothelial damage. These stimuli increases ADM production from pancreatic islets and vascular endothelium. Such a rise may represent a causative factor triggering the onset of disease and insulin resistance. If this assumption holds, a controlled reduction in ADM levels may improve hyperglycemia. To understand the casual role of ADM in diabetes, genetic variants could be a potential variable to study using Mendalian randomization, since it is unlikely to be confounded by environmental factors. Our recent study has demonstrated a positive link between a single nucleotide polymorphism (SNP) of ADM gene and development of dysglycemia[69]. Our other studies also demonstrates that plasma ADM level is associated with one of its SNP, IL-6 and adiponectin SNPs[70-72]. In the future regulation of ADM level could be a key in controlling glycemia in people with diabetes and this warrants further investigation.

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REFERENCES

1 **Hayashi M**, Shimosawa T, Isaka M, Yamada S, Fujita R, Fujita T. Plasma adrenomedullin in diabetes. *Lancet* 1997; **350**: 1449-1450 [PMID: 9371176 DOI: 10.1016/S0140-6736(05)64211-0]
2 **Yu CM**, Cheung BM, Leung R, Wang Q, Lai WH, Lau CP. Increase in plasma adrenomedullin in patients with heart failure characterised by diastolic dysfunction. *Heart* 2001; **86**: 155-160 [PMID: 11454830 DOI: 10.1136/heart.86.2.155]
3 **Wong HK**, Cheung TT, Cheung BM. Adrenomedullin and Cardiovascular Diseases. *J R Soc Med* 2012; **1**: 14 [PMID: 24175071]
4 **Martínez A**, Weaver C, López J, Bhathena SJ, Elsasser TH, Miller MJ, Moody TW, Unsworth EJ, Cuttitta F. Regulation of insulin secretion and blood glucose metabolism by adrenomedullin. *Endocrinology* 1996; **137**: 2626-2632 [PMID: 8641217]
5 **Kitamura K**, Kangawa K, Kawamoto M, Ichiki Y, Nakamura S, Matsuo H, Eto T. Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma. *Biochem Biophys Res Commun* 1993; **192**: 553-560 [PMID: 8387282 DOI: 10.1006/bbrc.1993.1451]
6 **Ichiki Y**, Kitamura K, Kangawa K, Kawamoto M, Matsuo H, Eto T. Distribution and characterization of immunoreactive adrenomedullin in human tissue and plasma. *FEBS Lett* 1994; **338**: 6-10 [PMID: 8307158 DOI: 10.1016/0014-5793(94)80106-1]
7 **Sugo S**, Minamino N, Kangawa K, Miyamoto K, Kitamura K, Sakata J, Eto T, Matsuo H. Endothelial cells actively synthesize and secrete adrenomedullin. *Biochem Biophys Res Commun* 1994; **201**: 1160-1166 [PMID: 8024557 DOI: 10.1006/bbrc.1994.1827]
8 **Ohta H**, Tsuji T, Asai S, Tanizaki S, Sasakura K, Teraoka H, Kitamura K, Kangawa K. A simple immunoradiometric assay for measuring the entire molecules of adrenomedullin in human plasma. *Clin Chim Acta* 1999; **287**: 131-143 [PMID: 10509902 DOI: 10.1016/S0009-8981(99)00128-X]
9 **Marutsuka K**, Hatakeyama K, Sato Y, Yamashita A, Sumiyoshi A, Asada Y. Immunohistological localization and possible functions of adrenomedullin. *Hypertens Res* 2003; **26** Suppl: S33-S40 [PMID: 12630809 DOI: 10.1291/hypres.26.S33]
10 **Kitamura K**, Sakata J, Kangawa K, Kojima M, Matsuo H, Eto T. Cloning and characterization of cDNA encoding a precursor for human adrenomedullin. *Biochem Biophys Res Commun* 1993; **194**: 720-725 [PMID: 7688224 DOI: 10.1006/bbrc.1993.1881]
11 **Hwang IS**, Tang F. Peripheral distribution and gene expression of adrenomedullin in the rat: possible source of blood adrenomedullin. *Neuropeptides* 2000; **34**: 32-37 [PMID: 10688966 DOI: 10.1054/npep.1999.0783]
12 **Sugo S**, Minamino N, Shoji H, Kangawa K, Kitamura K, Eto T, Matsuo H. Production and secretion of adrenomedullin from vascular smooth muscle cells: augmented production by tumor necrosis factor-alpha. *Biochem Biophys Res Commun* 1994; **203**: 719-726 [PMID: 8074727 DOI: 10.1006/bbrc.1994.2241]
13 **Martinez A**, Miller MJ, Unsworth EJ, Siegfried JM, Cuttitta F. Expression of adrenomedullin in normal human lung and in pulmonary tumors. *Endocrinology* 1995; **136**: 4099-4105 [PMID: 7649118]
14 **Satoh F**, Takahashi K, Murakami O, Totsune K, Sone M, Ohneda M, Abe K, Miura Y, Hayashi Y, Sasano H. Adrenomedullin in human brain, adrenal glands and tumor tissues of pheochromocytoma, ganglioneuroblastoma and neuroblastoma. *J Clin Endocrinol Metab* 1995; **80**: 1750-1752 [PMID: 7745031]
15 **Washimine H**, Asada Y, Kitamura K, Ichiki Y, Hara S, Yamamoto Y, Kangawa K, Sumiyoshi A, Eto T. Immunohistochemical identification of adrenomedullin in human, rat, and porcine tissue. *Histochem Cell Biol* 1995; **103**: 251-254 [PMID: 7648399 DOI: 10.1007/BF01457408]
16 **Sugo S**, Minamino N, Shoji H, Kangawa K, Kitamura K, Eto T, Matsuo H. Interleukin-1, tumor necrosis factor and lipopolysaccharide additively stimulate production of adrenomedullin in vascular smooth muscle cells. *Biochem Biophys Res Commun* 1995; **207**: 25-32 [PMID: 7857273 DOI: 10.1006/bbrc.1995.1148]
17 **Chun TH**, Itoh H, Ogawa Y, Tamura N, Takaya K, Igaki T, Yamashita J, Doi K, Inoue M, Masatsugu K, Korenaga R, Ando J, Nakao K. Shear stress augments expression of C-type natriuretic peptide and adrenomedullin. *Hypertension* 1997; **29**: 1296-1302 [PMID: 9180632 DOI: 10.1161/01.HYP.29.6.1296]
18 **Cheung BM**, Tang F. Adrenomedullin: exciting new horizons. *Recent Pat Endocr Metab Immune Drug Discov* 2012; **6**: 4-17 [PMID: 22216776 DOI: 10.2174/187221412799015263]
19 **Owji AA**, Smith DM, Coppock HA, Morgan DG, Bhogal R, Ghatei MA, Bloom SR. An abundant and specific binding site for the novel vasodilator adrenomedullin in the rat. *Endocrinology* 1995; **136**: 2127-2134 [PMID: 7720662]
20 **Hagner S**, Stahl U, Knoblauch B, McGregor GP, Lang RE. Calcitonin receptor-like receptor: identification and distribution in human peripheral tissues. *Cell Tissue Res* 2002; **310**: 41-50 [PMID: 12242482 DOI: 10.1007/s00441-002-0616-x]

21 **Poyner DR**, Sexton PM, Marshall I, Smith DM, Quirion R, Born W, Muff R, Fischer JA, Foord SM. International Union of Pharmacology. XXXII. The mammalian calcitonin gene-related peptides, adrenomedullin, amylin, and calcitonin receptors. *Pharmacol Rev* 2002; **54**: 233-246 [PMID: 12037140 DOI: 10.1124/pr.54.2.233]

22 **McLatchie LM**, Fraser NJ, Main MJ, Wise A, Brown J, Thompson N, Solari R, Lee MG, Foord SM. RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor. *Nature* 1998; **393**: 333-339 [PMID: 9620797 DOI: 10.1038/30666]

23 **Cheung BM**, Li CY, Wong LY. Adrenomedullin: its role in the cardiovascular system. *Semin Vasc Med* 2004; **4**: 129-134 [PMID: 15478033 DOI: 10.1055/s-2004-835370]

24 **Ishiyama Y**, Kitamura K, Ichiki Y, Nakamura S, Kida O, Kangawa K, Eto T. Hemodynamic effects of a novel hypotensive peptide, human adrenomedullin, in rats. *Eur J Pharmacol* 1993; **241**: 271-273 [PMID: 8243562 DOI: 10.1016/0014-2999(93)90214-3]

25 **He H**, Bessho H, Fujisawa Y, Horiuchi K, Tomohiro A, Kita T, Aki Y, Kimura S, Tamaki T, Abe Y. Effects of a synthetic rat adrenomedullin on regional hemodynamics in rats. *Eur J Pharmacol* 1995; **273**: 209-214 [PMID: 7737327 DOI: 10.1016/0014-2999(94)00683-X]

26 **Li YY**, Wong LY, Cheung BM, Hwang IS, Tang F. Differential induction of adrenomedullin, interleukins and tumour necrosis factor-alpha by lipopolysaccharide in rat tissues in vivo. *Clin Exp Pharmacol Physiol* 2005; **32**: 1110-1118 [PMID: 16445578 DOI: 10.1111/j.1440-1681.2005.04307.x]

27 **Dettmann ES**, Vysniauskiene I, Wu R, Flammer J, Haefliger IO. Adrenomedullin-induced endothelium-dependent relaxation in porcine ciliary arteries. *Invest Ophthalmol Vis Sci* 2003; **44**: 3961-3966 [PMID: 12939315 DOI: 10.1167/iovs.02-1312]

28 **Barber DA**, Park YS, Burnett JC, Miller VM. Adrenomedullin-mediated relaxations in veins are endothelium-dependent and distinct from arteries. *J Cardiovasc Pharmacol* 1997; **30**: 695-701 [PMID: 9388054 DOI: 10.1097/00005344-199711000-00022]

29 **Eguchi S**, Hirata Y, Kano H, Sato K, Watanabe Y, Watanabe TX, Nakajima K, Sakakibara S, Marumo F. Specific receptors for adrenomedullin in cultured rat vascular smooth muscle cells. *FEBS Lett* 1994; **340**: 226-230 [PMID: 8131850 DOI: 10.1016/0014-5793(94)80143-6]

30 **Hayakawa H**, Hirata Y, Kakoki M, Suzuki Y, Nishimatsu H, Nagata D, Suzuki E, Kikuchi K, Nagano T, Kangawa K, Matsuo H, Sugimoto T, Omata M. Role of nitric oxide-cGMP pathway in adrenomedullin-induced vasodilation in the rat. *Hypertension* 1999; **33**: 689-693 [PMID: 10024329 DOI: 10.1161/01.HYP.33.2.689]

31 **Allaker RP**, Grosvenor PW, McAnerney DC, Sheehan BE, Srikanta BH, Pell K, Kapas S. Mechanisms of adrenomedullin antimicrobial action. *Peptides* 2006; **27**: 661-666 [PMID: 16226342 DOI: 10.1016/j.peptides.2005.09.003]

32 **Allaker RP**, Kapas S. Adrenomedullin expression by gastric epithelial cells in response to infection. *Clin Diagn Lab Immunol* 2003; **10**: 546-551 [PMID: 12853384]

33 **Kubo A**, Minamino N, Isumi Y, Katafuchi T, Kangawa K, Dohi K, Matsuo H. Production of adrenomedullin in macrophage cell line and peritoneal macrophage. *J Biol Chem* 1998; **273**: 16730-16738 [PMID: 9642228 DOI: 10.1074/jbc.273.27.16730]

34 **Li YY**, Cheung BM, Wong LY, Hwang IS, Kumana CR, Tang F. Adrenomedullin gene expression and levels in the cardiovascular system after treatment with lipopolysaccharide. *Neuropeptides* 2005; **39**: 73-80 [PMID: 15752540 DOI: 10.1016/j.npep.2004.11.002]

35 **Cheung BM**, Hwang IS, Li CY, O WS, Tsang KW, Leung RY, Kumana CR, Tang F. Increased adrenomedullin expression in lungs in endotoxaemia. *J Endocrinol* 2004; **181**: 339-345 [PMID: 15128282 DOI: 10.1677/joe.0.1810339]

36 **Elsasser TH**, Kahl S. Adrenomedullin has multiple roles in disease stress: development and remission of the inflammatory response. *Microsc Res Tech* 2002; **57**: 120-129 [PMID: 11921363 DOI: 10.1002/jemt.10058]

37 **Liao SB**, Wong PF, Cheung BM, Tang F. Effects of adrenomedullin on tumour necrosis factor alpha, interleukins, endothelin-1, leptin, and adiponectin in the epididymal fat and soleus muscle of the rat. *Horm Metab Res* 2013; **45**: 31-37 [PMID: 22956308]

38 **Zudaire E**, Portal-Núñez S, Cuttitta F. The central role of adrenomedullin in host defense. *J Leukoc Biol* 2006; **80**: 237-244 [PMID: 16769769 DOI: 10.1189/jlb.0206123]

39 **Wong LY**, Cheung BM, Li YY, Tang F. Adrenomedullin is both proinflammatory and antiinflammatory: its effects on gene expression and secretion of cytokines and macrophage migration inhibitory factor in NR8383 macrophage cell line. *Endocrinology* 2005; **146**: 1321-1327 [PMID: 15576460 DOI: 10.1210/en.2004-1080]

40 **Nakayama M**, Takahashi K, Murakami O, Murakami H, Sasano H, Shirato K, Shibahara S. Adrenomedullin in monocytes and macrophages: possible involvement of macrophage-derived adrenomedullin in atherogenesis. *Clin Sci* (Lond) 1999; **97**: 247-251 [PMID: 10409481 DOI: 10.1042/CS19990108]

41 **Michibata H**, Mukoyama M, Tanaka I, Suga S, Nakagawa M, Ishibashi R, Goto M, Akaji K, Fujiwara Y, Kiso Y, Nakao K. Autocrine/paracrine role of adrenomedullin in cultured endothelial and mesangial cells. *Kidney Int* 1998; **53**: 979-985 [PMID: 9551407 DOI: 10.1111/j.1523-1755.1998.00855.x]

42 **Autelitano DJ**, Tang F, Little PJ. Rapid regulation of adrenomedullin in metabolically compromised vascular smooth muscle cells. *J Hypertens* 1999; **17**: 373-379 [PMID: 10100075 DOI: 10.1097/00004872-199917030-00010]

43 **Kano H**, Kohno M, Yasunari K, Yokokawa K, Horio T, Ikeda M, Minami M, Hanehira T, Takeda T, Yoshikawa J. Adrenomedullin as a novel antiproliferative factor of vascular smooth muscle cells. *J Hypertens* 1996; **14**: 209-213 [PMID: 8728298 DOI: 10.1097/00004872-199602000-00009]

44 **López J**, Cuesta N. Adrenomedullin as a pancreatic hormone. *Microsc Res Tech* 2002; **57**: 61-75 [PMID: 11921357 DOI: 10.1002/jemt.10049]

45 **Zudaire E**, Cuttitta F, Martínez A. Regulation of pancreatic physiology by adrenomedullin and its binding protein. *Regul Pept* 2003; **112**: 121-1.30 [PMID: 12667633 DOI: 10.1016/S0167-0115(03)00030-2]

46 Martı'nez A, Cuttitta F. Adrenomedullin, pancreatic islets, and diabetes. *Can J Diabetes Care* 2000; **24**: 39-46

47 **Tsuchida T**, Ohnishi H, Tanaka Y, Mine T, Fujita T. Inhibition of stimulated amylase secretion by adrenomedullin in rat pancreatic acini. *Endocrinology* 1999; **140**: 865-870 [PMID: 9927317 DOI: 10.1210/endo.140.2.6478]

48 **Bunton DC**, Petrie MC, Hillier C, Johnston F, McMurray JJ. The clinical relevance of adrenomedullin: a promising profile? *Pharmacol Ther* 2004; **103**: 179-201 [PMID: 15464589 DOI: 10.1016/j.pharmthera.2004.07.002]

49 **Wu JT**. Review of diabetes: identification of markers for early detection, glycemic control, and monitoring clinical complications. *J Clin Lab Anal* 1993; **7**: 293-300 [PMID: 8410489 DOI: 10.1002/jcla.1860070510]

50 **Hayashi M**, Shimosawa T, Fujita T. Hyperglycemia increases vascular adrenomedullin expression. *Biochem Biophys Res Commun* 1999; **258**: 453-456 [PMID: 10329408 DOI: 10.1006/bbrc.1999.0664]

51 **Tang F**, Hwang IS, Wong MP, Li YY. Adrenomedullin gene expression and peptide levels in the heart and blood vessels of streptozotocin-diabetic rats. *Horm Metab Res* 2007; **39**: 3-8 [PMID: 17226106 DOI: 10.1055/s-2007-956517]

52 **Kinoshita H**, Kato K, Kuroki M, Nakamura S, Kitamura K, Hisanaga S, Fujimoto S, Eto T. Plasma adrenomedullin levels in patients with diabetes. *Diabetes Care* 2000; **23**: 253-254 [PMID: 10868840 DOI: 10.2337/diacare.23.2.253]

53 **Cheung B**, Leung R. Elevated plasma levels of human adrenomedullin in cardiovascular, respiratory, hepatic and renal disorders. *Clin Sci* (Lond) 1997; **92**: 59-62 [PMID: 9038592]

54 **Ishimitsu T**, Nishikimi T, Saito Y, Kitamura K, Eto T, Kangawa K, Matsuo H, Omae T, Matsuoka H. Plasma levels of adrenomedullin, a newly identified hypotensive peptide, in patients with hypertension and renal failure. *J Clin Invest* 1994; **94**: 2158-2161 [PMID: 7962564 DOI: 10.1172/JCI117573]

55 **García-Unzueta MT**, Montalbán C, Pesquera C, Berrazueta JR, Amado JA. Plasma adrenomedullin levels in type 1 diabetes. Relationship with clinical parameters. *Diabetes Care* 1998; **21**: 999-1003 [PMID: 9614621 DOI: 10.2337/diacare.21.6.999]

56 **Hirayama N**, Kitamura K, Imamura T, Kato J, Koiwaya Y, Eto T. Secretion and clearance of the mature form of adrenomedullin in humans. *Life Sci* 1999; **64**: 2505-2509 [PMID: 10403510 DOI: 10.1016/S0024-3205(99)00208-8]

57 **Hiragushi K**, Wada J, Eguchi J, Matsuoka T, Yasuhara A, Hashimoto I, Yamashita T, Hida K, Nakamura Y, Shikata K, Minamino N, Kangawa K, Makino H. The role of adrenomedullin and receptors in glomerular hyperfiltration in streptozotocin-induced diabetic rats. *Kidney Int* 2004; **65**: 540-550 [PMID: 14717924 DOI: 10.1111/j.1523-1755.2004.00407.x]

58 **Ruzicska E**, Toth M, Tulassay Z, Somogyi A. Adrenomedullin and diabetes mellitus. *Diabetes Metab Res Rev* 2001; **17**: 321-329 [PMID: 11747138 DOI: 10.1002/dmrr.223]

59 **Kato H**, Shichiri M, Marumo F, Hirata Y. Adrenomedullin as an autocrine/paracrine apoptosis survival factor for rat endothelial cells. *Endocrinology* 1997; **138**: 2615-2620 [PMID: 9165056]

60 **Shimosawa T**, Shibagaki Y, Ishibashi K, Kitamura K, Kangawa K, Kato S, Ando K, Fujita T. Adrenomedullin, an endogenous peptide, counteracts cardiovascular damage. *Circulation* 2002; **105**: 106-111 [PMID: 11772884 DOI: 10.1161/hc0102.101399]

61 **Kato J**, Tsuruda T, Kita T, Kitamura K, Eto T. Adrenomedullin: a protective factor for blood vessels. *Arterioscler Thromb Vasc Biol* 2005; **25**: 2480-2487 [PMID: 16141406 DOI: 10.1161/01.ATV.0000184759.91369.f8]

62 **Nakamura T**, Honda K, Ishikawa S, Kitamura K, Eto T, Saito T. Plasma adrenomedullin levels in patients with non-insulin dependent diabetes mellitus: close relationships with diabetic complications. *Endocr J* 1998; **45**: 241-246 [PMID: 9700478 DOI: 10.1507/endocrj.45.241]

63 **Martínez A**, Elsasser TH, Bhathena SJ, Pío R, Buchanan TA, Macri CJ, Cuttitta F. Is adrenomedullin a causal agent in some cases of type 2 diabetes? *Peptides* 1999; **20**: 1471-1478 [PMID: 10698123 DOI: 10.1016/S0196-9781(99)00158-8]

64 **Lim SC**, Morgenthaler NG, Subramaniam T, Wu YS, Goh SK, Sum CF. The relationship between adrenomedullin, metabolic factors, and vascular function in individuals with type 2 diabetes. *Diabetes Care* 2007; **30**: 1513-1519 [PMID: 17384335 DOI: 10.2337/dc06-1899]

65 **Katsuki A**, Sumida Y, Gabazza EC, Murashima S, Urakawa H, Morioka K, Kitagawa N, Tanaka T, Araki-Sasaki R, Hori Y, Nakatani K, Yano Y, Adachi Y. Acute hyperinsulinemia is associated with increased circulating levels of adrenomedullin in patients with type 2 diabetes mellitus. *Eur J Endocrinol* 2002; **147**: 71-75 [PMID: 12088922 DOI: 10.1530/eje.0.1470071]

66 **Katsuki A**, Sumida Y, Urakawa H, Gabazza EC, Maruyama N, Morioka K, Kitagawa N, Hori Y, Nakatani K, Yano Y, Adachi Y. Increased oxidative stress is associated with elevated plasma levels of adrenomedullin in hypertensive patients with type 2 diabetes. *Diabetes Care* 2003; **26**: 1642-1643 [PMID: 12716843 DOI: 10.2337/diacare.26.5.1642]

67 **Chun TH**, Itoh H, Saito T, Yamahara K, Doi K, Mori Y, Ogawa Y, Yamashita J, Tanaka T, Inoue M, Masatsugu K, Sawada N, Fukunaga Y, Nakao K. Oxidative stress augments secretion of endothelium-derived relaxing peptides, C-type natriuretic peptide and adrenomedullin. *J Hypertens* 2000; **18**: 575-580 [PMID: 10826560 DOI: 10.1097/00004872-200018050-00010]

68 **Xing G**, Shimosawa T, Ogihara T, Matsui H, Itakura K, Qingyou X, Asano T, Ando K, Fujita T. Angiotensin II-induced insulin resistance is enhanced in adrenomedullin-deficient mice. *Endocrinology* 2004; **145**: 3647-3651 [PMID: 15105383 DOI: 10.1210/en.2004-0027]

69 **Ong KL**, Tso AW, Leung RY, Cherny SS, Sham PC, Lam TH, Cheung BM, Lam KS. A genetic variant in the gene encoding adrenomedullin predicts the development of dysglycemia over 6.4 years in Chinese. *Clin Chim Acta* 2011; **412**: 353-357 [PMID: 21075100 DOI: 10.1016/j.cca.2010.11.007]

70 **Cheung BM**, Ong KL, Tso AW, Leung RY, Cherny SS, Sham PC, Lam TH, Lam KS. Plasma adrenomedullin level is related to a single nucleotide polymorphism in the adrenomedullin gene. *Eur J Endocrinol* 2011; **165**: 571-577 [PMID: 21798961 DOI: 10.1530/EJE-11-0513]

71 **Wong HK**, Ong KL, Leung RY, Lam TH, Thomas GN, Lam KS, Cheung BM. A single nucleotide polymorphism of interleukin-6 gene is related to plasma adrenomedullin levels. *Clin Endocrinol* (Oxf) 2013; **79**: 504-509 [PMID: 23088295 DOI: 10.1111/cen.12078]

72 **Wong HK**, Ong KL, Leung RY, Cheung TT, Xu A, Lam TH, Lam KS, Cheung BM. Plasma level of adrenomedullin is influenced by a single nucleotide polymorphism in the adiponectin gene. *PLoS One* 2013; **8**: e70335 [PMID: 23936408 DOI: 10.1371/journal.pone.0070335]

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REVIEW

Diabetes and cancer: Associations, mechanisms, and implications for medical practice

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Abstract

Both diabetes mellitus and cancer are prevalent diseases worldwide. It is evident that there is a substantial increase in cancer incidence in diabetic patients. Epidemiologic studies have indicated that diabetic patients are at significantly higher risk of common cancers including pancreatic, liver, breast, colorectal, urinary tract, gastric and female reproductive cancers. Mortality due to cancer is moderately increased among patients with diabetes compared with those without. There is increasing evidence that some cancers are associated with diabetes, but the underlying mechanisms of this potential association have not been fully elucidated. Insulin is a potent growth factor that promotes cell proliferation and carcinogenesis directly and/or through insulin-like growth factor 1 (IGF-1). Hyperinsulinemia leads to an increase in the bioactivity of IGF-1 by inhibiting IGF binding protein-1. Hyperglycemia serves as a subordinate plausible explanation of carcinogenesis. High glucose may exert direct and indirect effects upon cancer cells to promote proliferation. Also chronic inflammation is considered as a hallmark of carcinogenesis. The multiple drugs involved in the treatment of diabetes seem to modify the risk of cancer. Screening to detect cancer at an early stage and appropriate treatment of diabetic patients with cancer are important to improve their prognosis. This paper summarizes the associations between diabetes and common cancers, interprets possible mechanisms involved, and addresses implications for medical practice.

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Key words: Diabetes mellitus; Cancer; Association; Mechanism; Medical practice

Core tip: The diabetes-cancer link is summarized and discussed in detail and it may potentially be attributed to hormonal disorders, chronic inflammation and metabolic alterations. Besides, implications for medical practice are also addressed.

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INTRODUCTION

The prevalence of diabetes mellitus (DM) is increasing worldwide. According to the estimates by the International Diabetes Federation, the global prevalence of type

2 diabetes mellitus (T2DM) is 8.3%. The prevalence of T2DM varies by country and area. The highest rate is 10.5% in North America, 8.7% in South-East Asia, 6.7% in Europe and 4.3% in Africa. It is predicted that 552 million people worldwide will develop diabetes by 2030[1].

DM and cancer are frequently diagnosed in the same individual[2]. DM is reported to be associated with an increased risk of different types of cancer, including pancreatic, liver, breast, colorectal, urinary tract, gastric, and female reproductive cancers. The relative risk ranges from 2.0 to 2.5 for liver, pancreatic and endometrial cancers, and 1.2 to 1.5 for breast, colon and bladder cancers associated with DM[3]. It is worth noting that DM is a growing health problem worldwide. Even if the increased risk in cancer incidence and mortality due to DM is small, the consequence would be significant at the population level[4].

The mechanism of DM associated with cancer remains uncovered and needs to be examined in further studies. The mechanism for the diabetes-cancer link has been hypothesized to be mainly related to hormonal [insulin and insulin-like growth factor (IGF)-1], inflammatory or metabolic (hyperglycemia) characteristics of the DM and even to certain treatments[5]. Anti-diabetic medications may have effects on the risk for cancer. Increasing evidence shows that insulin sensitizers such as metformin and thiazolidinediones (TZDs) are associated with prostate cancer[6] and HER2-positive breast cancer[7] among diabetic patients. The diabetic patients who are treated with insulin or insulin secretagogues are more likely to develop cancer than those with metformin[8-11].

In this paper, we summarize the associations between diabetes and cancer in epidemiologic studies, possible mechanisms and implications for medical practice.

POSSIBLE BIOLOGIC LINKS BETWEEN DIABETES AND CANCER RISK

Insulin resistance

Insulin resistance is very common in T2DM, in which circulating insulin level is frequently increased. The insulin/IGF axis plays an important role in diabetes-associated increased risk and progression of cancer. The cancer cells overexpress insulin and IGF-1 receptors[2].

Hyperinsulinemia is a hallmark of insulin resistance. The mechanisms whereby hyperinsulinemia could link diabetes and cancer have been extensively investigated and discussed. Hyperinsulinemia may influence cancer development through ligand by binding with the insulin receptor (IR) and/or indirectly through increasing circulating IGF-1 levels[12]. Insulin signal transduction is mediated through two IR isoforms: IR-A and IR-B[13]. IR-A recognizes insulin and IGFs, with a higher affinity for IGF2 than IGF1, and IR-B is insulin specific and is mainly involved in glucose homeostasis. Insulin binds with IR-A and exerts a direct pro-growth mitogenic effect. When elevated, insulin can increase the hepatic expression of IGF-1 and then activate the IGF-1 receptor, further stimulating cell growth through this mechanism[14,15]. IR-A and IGF-1 receptor are expressed primarily in fetal tissues and cancer cells[16].

The independent role of the IR is confirmed by the observation that down-regulation of IRs in LCC6 cells reduces xenograft tumor growth in athymic mice and inhibits lung metastasis[17]. Besides, blockade of the IGF-1 receptor has been associated with decreased growth of breast cancer cells[18,19]. Hyperinsulinemia also results in decreased levels of IGF binding protein-1 and thus increased levels of bioactive IGF-1[20,21].

Multiple downstream signaling pathways are activated after IRs or IGF-1 receptors interact with their ligands. By phosphorylation of adaptor proteins, two major pathways are involved: (1) the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt)/mammalian target of rapamycin (mTOR), PI3K/Akt/forkhead box O, and Ras/MAPK/extracellular signal-related kinase 1/2 pathway which plays important roles in cancer cell growth and carcinogenesis[22,23] is activated; and (2) the inhibitor of the oncogenic β-catenin signaling (glycogen synthase kinase 3β) is inactivated, through the PI3K/Akt signaling pathway, resulting in β-catenin signaling activation that has been related to cancer stem cells and chemoresistance[24].

Hyperglycemia

Hyperglycemia has been classically considered as a subordinate whereas hyperinsulinemia as a primary causal factor for cancer[25].

Several large cohort and case-control studies have found a positive relationship between hyperglycemia and the risk of cancer[26-29]. In a tumor-prone animal model, it was found that the number and size of liver tumors increased and apoptosis was reduced in insulin-deficient hyperglycemic mice compared with insulin-sufficient mice. This phenomenon was reversed by insulin therapy[30]. However, *in vivo* studies showed that T1DM, which is characterized by hyperglycemia, reduces the tumor growth. This finding does not support that hyperglycemia increases tumor growth, at least in the setting of insulin deficiency[31]. A recent research found that tumors continue to consume high amounts of glucose, regardless of plasma glucose levels[32]. A recent meta-analysis confirmed this finding that improved glycemic control does not reduce cancer risk in diabetic patients[33]. Hyperglycemia may be an independent risk factor for cancer. Further studies are needed to evaluate the relative roles of insulin and glucose.

The possible mechanisms of hyperglycemia increasing cancer risk include "indirect effect" and "direct effect"[34]. The "indirect effect" is the action that takes place at other organs and will later on influence tumor cells by inducing production of circulating growth factors (insulin/IGF-1) and inflammatory cytokines. The "direct effect" is the effect that is exerted directly upon tumor cells by increasing proliferation, inducing mutations, augmenting invasion and migration and rewiring cancer-related signaling pathways. Recently, Wnt/β-catenin signaling has been suggested as a key cancer-associated pathway and

Table 1 Combined relative risk and 95%CI in meta-analyses of cohort studies of cancer risk in different organs of diabetic patients

Cancer	Ref.	No. of cohort studies	RR (95%CI)	RR (95%CI) male	RR (95%CI) female
Pancreas	Ben *et al*[76], 2011	35	1.94 (1.66-2.27)	1.70 (1.55-1.87)[1]	1.60 (1.43-1.77)[1]
Liver	Wang *et al*[56], 2012	18	2.01 (1.61-2.51)	1.96 (1.71-2.24)[1]	1.66 (1.14-2.41)[1]
Breast	De Bruijn *et al*[66], 2013	20	1.23 (1.12-1.34)	NA	1.23 (1.12-1.34)
Endometrium	Zhang *et al*[67], 2013	15	1.81 (1.38-2.37)	NA	1.81 (1.38-2.37)
Colon-rectum	Jiang *et al*[62], 2011	30	1.27 (1.21-1.34)	1.25 (1.17-1.33)[1]	1.23 (1.13-1.33)[1]
Kidney	Bao *et al*[70], 2013	11	1.39 (1.09-1.78)	1.28 (1.10-1.48)	1.47 (1.18-1.73)
Bladder	Zhu *et al*[73], 2013	29	1.29 (1.08-1.54)	1.36 (1.05-1.77)	1.28 (0.75-2.19)
Prostate	Zhang *et al*[78], 2012	25	0.92 (0.81-1.05)	0.92 (0.81-1.05)	NA
Gastric	Yoon *et al*[81], 2013	11	1.20 (1.08-1.34)	1.10 (0.97-1.24)	1.24 (1.01-1.52)
Non-Hodgkin's lymphoma	Castillo *et al*[85], 2012	11	1.21 (1.02-1.45)	1.13 (0.96-1.34)	1.24 (0.97-1.58)

[1]Based on the studies reported by gender. NA: Unavailable.

high glucose enhances this signaling pathway by allowing nuclear retention and accumulation of transcriptionally active β-catenin independently of hyperinsulinemia, adipokines or inflammation[35,36].

Chronic inflammation

The deregulated metabolism in poorly controlled diabetes causes a long-term pro-inflammatory condition characterized by increased levels of interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), C-reactive protein, and other markers of chronic inflammation. Emerging evidence suggests that persistent inflammation can promote genetic instability and chronic inflammation is associated with increased cancer risk[37-40]. This finding is also supported by the classical evidence that non-steroidal anti-inflammatory drugs can reduce the risk of certain cancers[41-44].

Tumor-promoting mechanism of inflammation in diabetic patients is not much clear. Chronic inflammation and chronic oxidative stress go hand-in-hand. Oxidants affect almost all stages of the inflammatory response process, including the release of inflammatory cytokines, the sensing by innate immune receptors from the families of Toll-like receptors and the nucleotide-binding oligomerization domain-like receptors, and the activation of signaling initiating the adaptive cellular response to such signals[40]. Reactive oxygen species can cause damage to lipids, protein and DNA, and then initiate carcinogenesis[45-47]. Meanwhile, chronic inflammation is associated with high levels of TNF-α, which would strongly activate nuclear factor-kappa B (NF-κB) and further induce downstream signaling transduction to promote the development and progression of many tumors. NF-κB is involved in the proliferation and survival of malignant cells, promotes angiogenesis and metastasis, subverts adaptive immunity, and mediates responses to hormones and/or chemotherapeutic agents[48-50]. Therefore, continued exposure to chronic inflammation and oxidative stress puts susceptible cells at risk of progression toward malignant transformation[31].

IMPACT OF DIABETES ON CANCER

Evidence from animal studies

DM is mainly characterized by insulin resistance, hyperinsulinemia, hyperglycemia, and dyslipidemia. The independent role of diabetes and obesity in caner development has been difficult to distinguish since obesity is also related to inflammation and hyperinsulinemia. Studies in transgenic diabetic mice might shed light on the relative contributions of these factors. In a transgenic model of skin and mammary carcinogenesis, non-obese diabetic mice (A-ZIP/F-1) developed more tumors than wild-type controls[51]. In MKR mouse models of mammary carcinogenesis, female mice with T2DM showed accelerated mammary gland development and breast cancer progression independent of obesity and inflammation[52]. Hyperinsulinemia promoted the growth of primary mammary tumor and subsequent metastasis to the lung[53]. Tumor progression was abrogated with the decreased level of serum insulin after treatment with anti-insulin drugs[54]. Taken together, findings from animal studies support that diabetes plays interconnected roles in the development of cancer.

Epidemiologic findings

The findings from a meta-analysis of 12 cohort studies showed that diabetes increased the risk of all-cancer incidence for overall subjects, with a pooled adjusted RR of 1.14 (1.06-1.23) for men, and 1.18 (1.08-1.28) for women[55]. Diabetes is reported to be associated with several types of cancer, including pancreas, liver, breast, colorectal, urinary tract, gastric, and female reproductive cancers. Meta-analyses on the associations between diabetes and site specific cancer are summarized in Table 1.

Liver cancer: In various studies examining the link between DM and cancer, the highest risk has been seen for liver cancer. A meta-analysis demonstrated that individuals with diabetes had a 2.0-fold increased risk of developing hepatocellular carcinomas (HCC), compared with non-diabetics. And this link was observed in both men and women[56]. The liver is exposed to high concentrations of endogenously produced insulin transported *via* the portal vein. Hyperinsulinemia stimulates the production of IGF-1, which further promotes cellular proliferation and then inhibits apoptosis in the liver. The important role of hyperinsulinemia and IGF-1 in hepatic carcinogenesis has been demonstrated by *in vitro*, *in vivo*, and epidemiologic studies[57,58]. Liver steatosis, hepatitis,

and cirrhosis are more frequent among diabetic patients and are well known risk factors for HCC. Insulin resistance stimulates the release of multiple pro-inflammatory cytokines and consequently promotes the development of hepatic steatosis and inflammation and subsequent cancer in the liver[59]. A causal relationship was also reported by Jee *et al*[60], who found that fasting glucose and liver cancer risk had a dose-responsive relationship. Besides, T2DM-induced hyperglycemia induces the release of TNF-α and IL-6 in patients with hepatic steatosis and enhances the pathogenesis of cancer[61].

Colorectal cancer: A meta-analysis comprising 30 cohort studies showed that diabetes was associated with an increase in the risk of colorectal cancer, with a combined RR of 1.27 (1.21-1.34). This association was consistent for both men and women[62]. Our previous retrospective cohort study showed that a significant association of diabetes was found with colon cancer and not with rectal cancer[63]. This finding indicated that there was a subsite specific association of T2DM with colorectal cancer. General factors like hyperinsulinemia and IGF-1 have contributed to intramucosal adenocarcinomas. Diabetic patients have slower bowel peristalsis and more common constipation and thus increased exposure to bowel toxins (*i.e.*, elevated concentrations of fecal bile acids) and potential carcinogens[64]. Animal models have demonstrated that increased concentrations of fecal bile acids could induce colorectal carcinogenesis[64,65].

Breast and other female cancers: A meta-analysis including 20 cohort studies found an association between diabetes and breast cancer with a summary RR of 1.23 (1.12-1.34)[66]. A meta-analysis including 15 cohort studies reported an increased risk [RR = 1.81 (1.38-2.37)] of endometrial cancer in diabetic women[67]. Hyperinsulinemia could increase the levels of bioactive estrogens by reducing the concentration of circulating sex hormone binding protein in diabetic women. It is well known that bioactive estrogens are the risk factors for malignancies of female reproductive organs[68,69]. Increased bioactive estrogen will stimulate the proliferation of breast and endometrial cells and the inhibition of apoptosis to increase cancer risk.

Kidney and bladder cancers: A meta-analysis including eleven cohort studies showed that diabetes was significantly associated with an increased risk of kidney cancer [RR = 1.39 (1.09-1.78)]. The association was slightly stronger in women [RR = 1.47 (1.18-1.83)] than in men [RR = 1.28 (1.10-1.48)][70]. Hypertension and late stage renal disease, two common comorbidities of DM, contribute to the increased incidence of kidney cancer[71,72]. Impaired renal function results in higher circulating levels of carcinogens and toxins and immune inhibition and thereby renders the kidney susceptible to carcinogens and tumor growth. Findings from a meta-analysis of 29 cohort studies suggest that individuals with DM display an increase in the risk of bladder cancer [RR = 1.29 (1.08-1.54)]. The positive association is only observed in men [RR = 1.36 (1.05-1.77)][73]. In addition to general factors, the frequent infections of the urinary tract in diabetic patients might also be involved[74].

Pancreatic cancer: In a 3-year follow-up study[75], subjects with new-onset DM had a higher risk of pancreatic cancer with a RR of 7.94 than the subjects without DM. A meta-analysis of 35 cohort studies showed that DM was associated with an increased risk of pancreatic cancer in both men and women[76]. However, the question arises about whether diabetes is a risk factor or the consequence of the pancreatic cancer (so-called "reverse causality"). Pancreatic cancer might induce a diabetic status because of impaired pancreatic beta cells. *In vitro* studies show that blockage of insulin receptors and impaired insulin action and glucose transport in a model of pancreatic cancer led to insulin resistance[77]. However, the new onset of pancreatic cancer induced DM depends on the peripheral insulin resistance rather than on the impaired pancreatic beta cells. On the other hand, in patients with T2DM exocrine pancreatic cells are exposed to very high insulin levels because of their proximity to insulin secreting islets. Insulin stimulates the growth of cancer cells. Thus, hyperinsulinemia might account for the risk of developing pancreatic cancer in T2DM.

Prostate cancer: Prostate cancer risk appears to decrease in patients with diabetes. An inverse association was observed between diabetes and risk of prostate cancer in the studies from the United States but not in the studies from other countries, as shown by an updated meta-analysis[78]. The protective effect of DM was also observed in different grades or stages of prostate cancer in another meta-analysis[79]. One possible explanation is that low testosterone levels have been shown in diabetic men. The conversion of testosterone to dihydrotestosterone promotes prostate cell growth[80].

Other cancers in diabetes: A 20% increased gastric cancer risk in diabetic patients was found in a meta-analysis. A positive association was observed in female diabetic patients, whereas it was not the case in diabetic men[81]. The IGF/IGF-IR axis interacts with the vascular endothelial growth factor/vascular endothelial growth factor receptor system in gastrointestinal malignancies[82,83]. It is also possible that reactive oxygen-dependent DNA damage further enhances the effect of *Helicobacter pylori* on epithelial cell proliferation[84]. A meta-analysis of large prospective cohort studies has shown a moderate increase of non-Hodgkin's lymphoma in diabetic patients, whereas stratified analysis by gender shows no significance based on the studies with reported cancer incidence by gender[85]. The immune dysfunction related to impaired neutrophil activity and abnormalities in cellular and humoral immunity in diabetes may contribute to cancer development[86].

MORTALITY

A meta-analysis suggests that preexisting diabetes is as-

Table 2 Pooled HRs and 95%CI of all-cause mortality in cancer patients with and without preexisting diabetes mellitus

Cancer	Ref.	No. of cohort studies	HR (95%CI)	HR (95%CI) male	HR (95%CI) female
Pancreas	Barone *et al*[87], 2008	4	1.09 (0.70-1.69)	NA	NA
Liver	Wang *et al*[56], 2012	3	1.56 (1.30-1.87)	1.84 (1.34-2.51)	1.31 (1.06-1.61)
Breast	De Bruijn *et al*[66], 2013	20	1.38 (1.20-1.58)	NA	1.38 (1.20-1.58)
Endometrium	Zhang *et al*[67], 2013	6	1.23 (0.80-1.90)	NA	1.23 (0.80-1.90)
Colon-rectum	Jiang *et al*[62], 2011	11	1.20 (1.03-1.40)	1.26 (1.04-1.52)	1.18 (0.98-1.41)
Kidney	Bao *et al*[70], 2013	8	1.12 (0.99-1.20)	NA	NA
Bladder	Zhu *et al*[73], 2013	11	1.33 (1.14-1.55)	1.54 (1.30-1.82)[1]	1.50 (1.05-2.14)[1]
Prostate	Barone *et al*[87], 2008	3	1.51 (0.94-2.43)	1.51 (0.94-2.43)	NA
Gastric	Tian *et al*[88], 2012	NA	1.29 (1.04-1.59)	NA	NA
Non-Hodgkin's lymphoma	Lin *et al*[89], 2007	1	1.33 (0.61-2.90)	NA	NA

[1]Based on the studies reported by gender. NA: Unavailable.

sociated with a higher risk of all-cause long term cancer mortality compared with non-diabetic individuals HR = 1.41 (1.28-1.55)[87]. Mortality among diabetes was significantly increased for liver, breast, and bladder cancers, with pooled RRs of 1.56 (1.30-1.87)[56], 1.38 (1.20-1.58)[66], and 1.33 (1.14-1.55)[73], respectively. Similar but mild results are also seen in gastric cancer[88] and colorectal cancer[62]; with 29% and 20% increased all-cause mortalities, respectively (Table 2). Non-significance is found for the cancers of the pancreas[87], prostate[87], kidney[70], endometrium[67], and non-Hodgkin's lymphoma[89] (Table 2).

Several possible explanations might elucidate the increased risk of cancer death in DM. Impaired immune function and pro-inflammatory condition in diabetes may make the cancer more aggressive, favor cancer growth by making host organism less resistant to cancer progression, and strengthen the metastatic potential of cancer. Hyperglycemia may be an important risk factor. There is evidence that poor glycemic controls can lead to poorer outcomes. Survival rates in cancer are decreasing linearly with declining glycemic controls[90]. Diabetic patients may have a worse response to chemotherapy with a higher occurrence of adverse effects compared with non-diabetic individuals.

Diabetes patients are more often poor candidates for surgery. Preexisting diabetes was associated with increased odds of postoperative mortality across all cancer types [OR = 1.51 (1.13-2.02)][91].

IMPLICATIONS FOR MEDICAL PRACTICE

Cancer screening is required for patients with preexisting diabetes

As shown by the above studies, patients with DM have a higher risk of developing certain types of cancer. A healthy diet, physical activity, and weight management could decrease the risk and improve outcomes of DM and some types of cancer. This was supported by a consensus report of the American Diabetes Association and the American Cancer Society[2]. In order to improve the prognosis, early screening of DM-related cancers is important for T2DM patients. Cancer screening tests of proven benefit for malignancies (breast, colon, endometrial cancer, *etc.*) in at-risk individuals/populations should begin relatively earlier than the general population. Future cancer screenings should be based on current existing recommendations. However, specific DM-related cancer screening recommendations remain to be made.

The impact of anti-diabetic treatments on cancer risk

The major classes of DM drugs function to replace circulating insulin and reduce hyperglycemia by different mechanisms or to reduce the associated obesity[92]. Insulin sensitizers, including metformin and TZDs, are oral antidiabetic drugs that decrease insulin resistance by altering signaling through the AKT/mTOR pathway[93,94].

Metformin has been used with confidence in the treatment of T2DM[95]. Emerging evidence from research on humans and from the preclinical setting suggests that metformin has an anti-cancer effect. A meta-analysis of 17 randomized controlled trials showed a clinically significant 39% decreased risk of cancer with metformin use in patients with or at risk for diabetes, compared to no use of metformin[96]. Metformin can decrease cell proliferation and induce apoptosis in certain cancer cell lines[97,98]. In a recent retrospective cohort study, metformin use is not associated with improved survival in subjects with advanced pancreatic cancer[99]. Whereas metformin use was also reported to be associated with a lower risk of colon, liver, pancreas, or breast cancers, it was not associated with the risk of prostate cancer[100,101]. In a meta-analysis by Colmers *et al*[102], TZD-based therapy has been associated with a potential cancer risk, primarily pioglitazone with bladder cancer, as well as a protective role in breast, lung, and colorectal cancers. In combination, the majority of studies showed that metformin therapy decreases and insulin and insulin secretagogues slightly increase the risk of certain cancers in T2DM. Nonetheless, it is premature to prescribe metformin and TZDs solely for those as yet unproven indications for cancers.

Managing diabetic patients with cancer

Managing diabetes can be a daunting task for patients with cancer. Diabetes may negatively impact both cancer risk and outcomes of cancer treatment. It is clear that comorbidities may play a role in clinical outcomes in patients with cancer. Clinicians who treat cancer patients with T2DM should pay more attention to comorbidi-

ties. Thus, rigorous and multifactorial approaches should be adopted to control diabetes for patients undergoing treatment for malignancies. Poor glycemic control increases morbidity and mortality in patients with cancer. Therefore, hyperglycemia management in patients with cancer is important. Monitoring symptoms of both hyperglycemia and hypoglycemia is necessary. DM patients with cancer and their family members should monitor these symptoms and render suitable medical treatment once these symptoms occur. For hospitalized patients with acute concurrent complications, aggressive glycemic management should be taken to improve the prognosis.

CONCLUSION

Previous evidence provides strong support for an increase of both cancer risk and mortality in diabetic patients and more evidence for certain site-specific cancers. The molecular mechanisms for the association between diabetes and cancer development are still uncovered. As underlined in this review, mechanisms on hormonal (insulin and IGF-1), inflammatory and metabolic (hyperglycemia) characteristics have been proposed to elucidate this association. Guidelines specific for diabetic patients should include both treatment in medical practices and mass screening for specific cancers according to the risk factor profile of each patient.

REFERENCES

1 IDF Diabetes Atlas. International Diabetes Federation. Available from URL: http: //www.idf.org/diabetesatlas

2 **Onitilo AA**, Engel JM, Glurich I, Stankowski RV, Williams GM, Doi SA. Diabetes and cancer I: risk, survival, and implications for screening. *Cancer Causes Control* 2012; **23**: 967-981 [PMID: 22552844 DOI: 10.1007/s10552-012-9972-3]

3 **Giovannucci E**, Harlan DM, Archer MC, Bergenstal RM, Gapstur SM, Habel LA, Pollak M, Regensteiner JG, Yee D. Diabetes and cancer: a consensus report. *CA Cancer J Clin* 2010; **60**: 207-221 [PMID: 20554718 DOI: 10.3322/caac.20078]

4 **Sciacca L**, Vigneri R, Tumminia A, Frasca F, Squatrito S, Frittitta L, Vigneri P. Clinical and molecular mechanisms favoring cancer initiation and progression in diabetic patients. *Nutr Metab Cardiovasc Dis* 2013; **23**: 808-815 [PMID: 23932729 DOI: 10.1016/j.numecd.2013.05.006]

5 **García-Jiménez C**, García-Martínez JM, Chocarro-Calvo A, De la Vieja A. A new link between diabetes and cancer: enhanced WNT/β-catenin signaling by high glucose. *J Mol Endocrinol* 2014; **52**: R51-R66 [PMID: 24049067 DOI: 10.1530/JME-13-0152]

6 **He XX**, Tu SM, Lee MH, Yeung SC. Thiazolidinediones and metformin associated with improved survival of diabetic prostate cancer patients. *Ann Oncol* 2011; **22**: 2640-2645 [PMID: 21415239 DOI: 10.1093/annonc/mdr020]

7 **Martin-Castillo B**, Dorca J, Vazquez-Martin A, Oliveras-Ferraros C, Lopez-Bonet E, Garcia M, Del Barco S, Menendez JA. Incorporating the antidiabetic drug metformin in HER2-positive breast cancer treated with neo-adjuvant chemotherapy and trastuzumab: an ongoing clinical-translational research experience at the Catalan Institute of Oncology. *Ann Oncol* 2010; **21**: 187-189 [PMID: 19884247 DOI: 10.1093/annonc/mdp494]

8 **Evans JM**, Donnelly LA, Emslie-Smith AM, Alessi DR, Morris AD. Metformin and reduced risk of cancer in diabetic patients. *BMJ* 2005; **330**: 1304-1305 [PMID: 15849206 DOI: 10.1136/bmj.38415.708634.F7]

9 **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]

10 **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]

11 **Bowker SL**, Majumdar SR, Veugelers P, Johnson JA. Increased cancer-related mortality for patients with type 2 diabetes who use sulfonylureas or insulin: Response to Farooki and Schneider. *Diabetes Care* 2006; **29**: 1990-1991 [PMID: 16873829 DOI: 10.2337/dc06-0997]

12 **Roberts DL**, Dive C, Renehan AG. Biological mechanisms linking obesity and cancer risk: new perspectives. *Annu Rev Med* 2010; **61**: 301-316 [PMID: 19824817 DOI: 10.1146/annurev.med.080708.082713]

13 **Chettouh H**, Fartoux L, Aoudjehane L, Wendum D, Claperon A, Chrétien Y, Rey C, Scatton O, Soubrane O, Conti F, Praz F, Housset C, Rosmorduc O, Desbois-Mouthon C. Mitogenic insulin receptor-A is overexpressed in human hepatocellular carcinoma due to EGFR-mediated dysregulation of RNA splicing factors. *Cancer Res* 2013; **73**: 3974-3986 [PMID: 23633480 DOI: 10.1158/0008-5472.CAN-12-3824]

14 **Djiogue S**, Nwabo Kamdje AH, Vecchio L, Kipanyula MJ, Farahna M, Aldebasi Y, Seke Etet PF. Insulin resistance and cancer: the role of insulin and IGFs. *Endocr Relat Cancer* 2013; **20**: R1-R17 [PMID: 23207292 DOI: 10.1530/ERC-12-0324]

15 **Novosyadlyy R**, LeRoith D. Hyperinsulinemia and type 2 diabetes: impact on cancer. *Cell Cycle* 2010; **9**: 1449-1450 [PMID: 20372078 DOI: 10.4161/cc.9.8.11512]

16 **Frasca F**, Pandini G, Scalia P, Sciacca L, Mineo R, Costantino A, Goldfine ID, Belfiore A, Vigneri R. Insulin receptor isoform A, a newly recognized, high-affinity insulin-like growth factor II receptor in fetal and cancer cells. *Mol Cell Biol* 1999; **19**: 3278-3288 [PMID: 10207053]

17 **Zhang H**, Fagan DH, Zeng X, Freeman KT, Sachdev D, Yee D. Inhibition of cancer cell proliferation and metastasis by insulin receptor downregulation. *Oncogene* 2010; **29**: 2517-2527 [PMID: 20154728 DOI: 10.1038/onc.2010.17]

18 **Arteaga CL**, Kitten LJ, Coronado EB, Jacobs S, Kull FC, Allred DC, Osborne CK. Blockade of the type I somatomedin receptor inhibits growth of human breast cancer cells in athymic mice. *J Clin Invest* 1989; **84**: 1418-1423 [PMID: 2553774 DOI: 10.1172/JCI114315]

19 **Arteaga CL**, Osborne CK. Growth inhibition of human breast cancer cells in vitro with an antibody against the type I somatomedin receptor. *Cancer Res* 1989; **49**: 6237-6241 [PMID: 2553250]

20 **Levine AJ**, Feng Z, Mak TW, You H, Jin S. Coordination and communication between the p53 and IGF-1-AKT-TOR signal transduction pathways. *Genes Dev* 2006; **20**: 267-275 [PMID: 16452501 DOI: 10.1101/gad.1363206]

21 **Qin L**, Wang Y, Tao L, Wang Z. AKT down-regulates insulin-like growth factor-1 receptor as a negative feedback. *J Biochem* 2011; **150**: 151-156 [PMID: 21616916 DOI: 10.1093/jb/mvr066]

22 **Alvino CL**, Ong SC, McNeil KA, Delaine C, Booker GW, Wallace JC, Forbes BE. Understanding the mechanism of insulin and insulin-like growth factor (IGF) receptor activation by IGF-II. *PLoS One* 2011; **6**: e27488 [PMID: 22140443 DOI: 10.1371/journal.pone.0027488]

23 **Tzivion G**, Dobson M, Ramakrishnan G. FoxO transcription factors; Regulation by AKT and 14-3-3 proteins. *Biochim Biophys Acta* 2011; **1813**: 1938-1945 [PMID: 21708191 DOI: 10.1016/j.bbamcr.2011.06.002]

24 **Fleming HE**, Janzen V, Lo Celso C, Guo J, Leahy KM, Kronenberg HM, Scadden DT. Wnt signaling in the niche en-

forces hematopoietic stem cell quiescence and is necessary to preserve self-renewal in vivo. *Cell Stem Cell* 2008; **2**: 274-283 [PMID: 18371452 DOI: 10.1016/j.stem.2008.01.003]
25 **Giovannucci E**. Insulin, insulin-like growth factors and colon cancer: a review of the evidence. *J Nutr* 2001; **131**: 3109S-3120S [PMID: 11694656]
26 **Muti P**, Quattrin T, Grant BJ, Krogh V, Micheli A, Schünemann HJ, Ram M, Freudenheim JL, Sieri S, Trevisan M, Berrino F. Fasting glucose is a risk factor for breast cancer: a prospective study. *Cancer Epidemiol Biomarkers Prev* 2002; **11**: 1361-1368 [PMID: 12433712]
27 **Saydah SH**, Platz EA, Rifai N, Pollak MN, Brancati FL, Helzlsouer KJ. Association of markers of insulin and glucose control with subsequent colorectal cancer risk. *Cancer Epidemiol Biomarkers Prev* 2003; **12**: 412-418 [PMID: 12750235]
28 **Stattin P**, Björ O, Ferrari P, Lukanova A, Lenner P, Lindahl B, Hallmans G, Kaaks R. Prospective study of hyperglycemia and cancer risk. *Diabetes Care* 2007; **30**: 561-567 [PMID: 17327321 DOI: 10.2337/dc06-0922]
29 **Takahashi H**, Mizuta T, Eguchi Y, Kawaguchi Y, Kuwashiro T, Oeda S, Isoda H, Oza N, Iwane S, Izumi K, Anzai K, Ozaki I, Fujimoto K. Post-challenge hyperglycemia is a significant risk factor for the development of hepatocellular carcinoma in patients with chronic hepatitis C. *J Gastroenterol* 2011; **46**: 790-798 [PMID: 21331763 DOI: 10.1007/s00535-011-0381-2]
30 **Yamasaki K**, Hayashi Y, Okamoto S, Osanai M, Lee GH. Insulin-independent promotion of chemically induced hepatocellular tumor development in genetically diabetic mice. *Cancer Sci* 2010; **101**: 65-72 [PMID: 19775285 DOI: 10.1111/j.1349-7006.2009.01345.x]
31 **Vigneri P**, Frasca F, Sciacca L, Pandini G, Vigneri R. Diabetes and cancer. *Endocr Relat Cancer* 2009; **16**: 1103-1123 [PMID: 19620249 DOI: 10.1677/ERC-09-0087]
32 **Taubes G**. Cancer research. Unraveling the obesity-cancer connection. *Science* 2012; **335**: 28, 30-32 [PMID: 22223787 DOI: 10.1126/science.335.6064.28]
33 **Johnson JA**, Bowker SL. Intensive glycaemic control and cancer risk in type 2 diabetes: a meta-analysis of major trials. *Diabetologia* 2011; **54**: 25-31 [PMID: 20959956 DOI: 10.1007/s00125-010-1933-3]
34 **Ward PS**, Thompson CB. Metabolic reprogramming: a cancer hallmark even warburg did not anticipate. *Cancer Cell* 2012; **21**: 297-308 [PMID: 22439925 DOI: 10.1016/j.ccr.2012.02.014]
35 **Chocarro-Calvo A**, García-Martínez JM, Ardila-González S, De la Vieja A, García-Jiménez C. Glucose-induced β-catenin acetylation enhances Wnt signaling in cancer. *Mol Cell* 2013; **49**: 474-486 [PMID: 23273980 DOI: 10.1016/j.molcel.2012.11.022]
36 **Anagnostou SH**, Shepherd PR. Glucose induces an autocrine activation of the Wnt/beta-catenin pathway in macrophage cell lines. *Biochem J* 2008; **416**: 211-218 [PMID: 18823284 DOI: 10.1042/BJ20081426]
37 **Kundu JK**, Surh YJ. Inflammation: gearing the journey to cancer. *Mutat Res* 2008; **659**: 15-30 [PMID: 18485806 DOI: 10.1016/j.mrrev.2008.03.002]
38 **Ono M**. Molecular links between tumor angiogenesis and inflammation: inflammatory stimuli of macrophages and cancer cells as targets for therapeutic strategy. *Cancer Sci* 2008; **99**: 1501-1506 [PMID: 18754859 DOI: 10.1111/j.1349-7006.2008.00853.x]
39 **Moore MM**, Chua W, Charles KA, Clarke SJ. Inflammation and cancer: causes and consequences. *Clin Pharmacol Ther* 2010; **87**: 504-508 [PMID: 20147899 DOI: 10.1038/clpt.2009.254]
40 **Del Prete A**, Allavena P, Santoro G, Fumarulo R, Corsi MM, Mantovani A. Molecular pathways in cancer-related inflammation. *Biochem Med* (Zagreb) 2011; **21**: 264-275 [PMID: 22420240]
41 **Brasky TM**, Potter JD, Kristal AR, Patterson RE, Peters U, Asgari MM, Thornquist MD, White E. Non-steroidal anti-inflammatory drugs and cancer incidence by sex in the VITamins And Lifestyle (VITAL) cohort. *Cancer Causes Control* 2012; **23**: 431-444 [PMID: 22212612 DOI: 10.1007/s10552-011-9891-8]
42 **Murphy MA**, Trabert B, Yang HP, Park Y, Brinton LA, Hartge P, Sherman ME, Hollenbeck A, Wentzensen N. Non-steroidal anti-inflammatory drug use and ovarian cancer risk: findings from the NIH-AARP Diet and Health Study and systematic review. *Cancer Causes Control* 2012; **23**: 1839-1852 [PMID: 22972000 DOI: 10.1007/s10552-012-0063-2]
43 **Setiawan VW**, Matsuno RK, Lurie G, Wilkens LR, Carney ME, Henderson BE, Kolonel LN, Goodman MT. Use of non-steroidal anti-inflammatory drugs and risk of ovarian and endometrial cancer: the Multiethnic Cohort. *Cancer Epidemiol Biomarkers Prev* 2012; **21**: 1441-1449 [PMID: 22665579 DOI: 10.1158/1055-9965.EPI-12-0390-T]
44 **Silva MT**, Galvao TF, Zimmerman IR, Pereira MG, Lopes LC. Non-aspirin non-steroidal anti-inflammatory drugs for the primary chemoprevention of non-gastrointestinal cancer: summary of evidence. *Curr Pharm Des* 2012; **18**: 4047-4070 [PMID: 22632754 DOI: 10.2174/138161212802083699]
45 **Pitocco D**, Zaccardi F, Di Stasio E, Romitelli F, Santini SA, Zuppi C, Ghirlanda G. Oxidative stress, nitric oxide, and diabetes. *Rev Diabet Stud* 2010; **7**: 15-25 [PMID: 20703435 DOI: 10.1900/RDS.2010.7.15]
46 **Matsuda M**, Shimomura I. Increased oxidative stress in obesity: implications for metabolic syndrome, diabetes, hypertension, dyslipidemia, atherosclerosis, and cancer. *Obes Res Clin Pract* 2013; **7**: e330-e341 [PMID: 24455761]
47 **Yang H**, Jin X, Kei Lam CW, Yan SK. Oxidative stress and diabetes mellitus. *Clin Chem Lab Med* 2011; **49**: 1773-1782 [PMID: 21810068 DOI: 10.1515/CCLM.2011.250]
48 **DiDonato JA**, Mercurio F, Karin M. NF-κB and the link between inflammation and cancer. *Immunol Rev* 2012; **246**: 379-400 [PMID: 22435567 DOI: 10.1111/j.1600-065X.2012.01099.x]
49 **Hoesel B**, Schmid JA. The complexity of NF-κB signaling in inflammation and cancer. *Mol Cancer* 2013; **12**: 86 [PMID: 23915189 DOI: 10.1186/1476-4598-12-86]
50 **Karin M**. NF-kappaB as a critical link between inflammation and cancer. *Cold Spring Harb Perspect Biol* 2009; **1**: a000141 [PMID: 20066113 DOI: 10.1101/cshperspect.a000141]
51 **Nunez NP**, Oh WJ, Rozenberg J, Perella C, Anver M, Barrett JC, Perkins SN, Berrigan D, Moitra J, Varticovski L, Hursting SD, Vinson C. Accelerated tumor formation in a fatless mouse with type 2 diabetes and inflammation. *Cancer Res* 2006; **66**: 5469-5476 [PMID: 16707476 DOI: 10.1158/0008-5472.CAN-05-4102]
52 **Novosyadlyy R**, Lann DE, Vijayakumar A, Rowzee A, Lazzarino DA, Fierz Y, Carboni JM, Gottardis MM, Pennisi PA, Molinolo AA, Kurshan N, Mejia W, Santopietro S, Yakar S, Wood TL, LeRoith D. Insulin-mediated acceleration of breast cancer development and progression in a nonobese model of type 2 diabetes. *Cancer Res* 2010; **70**: 741-751 [PMID: 20068149 DOI: 10.1158/0008-5472.CAN-09-2141]
53 **Ferguson RD**, Novosyadlyy R, Fierz Y, Alikhani N, Sun H, Yakar S, Leroith D. Hyperinsulinemia enhances c-Myc-mediated mammary tumor development and advances metastatic progression to the lung in a mouse model of type 2 diabetes. *Breast Cancer Res* 2012; **14**: R8 [PMID: 22226054 DOI: 10.1186/bcr3089]
54 **Fierz Y**, Novosyadlyy R, Vijayakumar A, Yakar S, LeRoith D. Insulin-sensitizing therapy attenuates type 2 diabetes-mediated mammary tumor progression. *Diabetes* 2010; **59**: 686-693 [PMID: 19959755 DOI: 10.2337/db09-1291]
55 **Noto H**, Tsujimoto T, Sasazuki T, Noda M. Significantly increased risk of cancer in patients with diabetes mellitus: a systematic review and meta-analysis. *Endocr Pract* 2011; **17**: 616-628 [PMID: 21454235 DOI: 10.4158/EP10357.RA]
56 **Wang C**, Wang X, Gong G, Ben Q, Qiu W, Chen Y, Li G, Wang L. Increased risk of hepatocellular carcinoma in pa-

tients with diabetes mellitus: a systematic review and meta-analysis of cohort studies. *Int J Cancer* 2012; **130**: 1639-1648 [PMID: 21544812 DOI: 10.1002/ijc.26165]
57 **Weng CJ**, Hsieh YH, Tsai CM, Chu YH, Ueng KC, Liu YF, Yeh YH, Su SC, Chen YC, Chen MK, Yang SF. Relationship of insulin-like growth factors system gene polymorphisms with the susceptibility and pathological development of hepatocellular carcinoma. *Ann Surg Oncol* 2010; **17**: 1808-1815 [PMID: 20119675 DOI: 10.1245/s10434-009-0904-8]
58 **Wiencke JK**. Impact of race/ethnicity on molecular pathways in human cancer. *Nat Rev Cancer* 2004; **4**: 79-84 [PMID: 14708028 DOI: 10.1038/nrc1257]
59 **Papa S**, Bubici C, Zazzeroni F, Franzoso G. Mechanisms of liver disease: cross-talk between the NF-kappaB and JNK pathways. *Biol Chem* 2009; **390**: 965-976 [PMID: 19642868 DOI: 10.1515/BC.2009.111]
60 **Jee SH**, Ohrr H, Sull JW, Yun JE, Ji M, Samet JM. Fasting serum glucose level and cancer risk in Korean men and women. *JAMA* 2005; **293**: 194-202 [PMID: 15644546]
61 **Shams ME**, Al-Gayyar MM, Barakat EA. Type 2 Diabetes Mellitus-Induced Hyperglycemia in Patients with NAFLD and Normal LFTs: Relationship to Lipid Profile, Oxidative Stress and Pro-Inflammatory Cytokines. *Sci Pharm* 2011; **79**: 623-634 [PMID: 21886908 DOI: 10.3797/scipharm.1104-21]
62 **Jiang Y**, Ben Q, Shen H, Lu W, Zhang Y, Zhu J. Diabetes mellitus and incidence and mortality of colorectal cancer: a systematic review and meta-analysis of cohort studies. *Eur J Epidemiol* 2011; **26**: 863-876 [PMID: 21938478 DOI: 10.1007/s10654-011-9617-y]
63 **Ren X**, Zhang X, Zhang X, Gu W, Chen K, Le Y, Lai M, Zhu Y. Type 2 diabetes mellitus associated with increased risk for colorectal cancer: evidence from an international ecological study and population-based risk analysis in China. *Public Health* 2009; **123**: 540-544 [PMID: 19664792 DOI: 10.1016/j.puhe.2009.06.019]
64 **Kajiura K**, Ohkusa T, Okayasu I. Relationship between fecal bile acids and the occurrence of colorectal neoplasia in experimental murine ulcerative colitis. *Digestion* 1998; **59**: 69-72 [PMID: 9468101]
65 **Debruyne PR**, Bruyneel EA, Li X, Zimber A, Gespach C, Mareel MM. The role of bile acids in carcinogenesis. *Mutat Res* 2001; **480-481**: 359-369 [PMID: 11506828]
66 **De Bruijn KM**, Arends LR, Hansen BE, Leeflang S, Ruiter R, van Eijck CH. Systematic review and meta-analysis of the association between diabetes mellitus and incidence and mortality in breast and colorectal cancer. *Br J Surg* 2013; **100**: 1421-1429 [PMID: 24037561 DOI: 10.1002/bjs.9229]
67 **Zhang ZH**, Su PY, Hao JH, Sun YH. The role of preexisting diabetes mellitus on incidence and mortality of endometrial cancer: a meta-analysis of prospective cohort studies. *Int J Gynecol Cancer* 2013; **23**: 294-303 [PMID: 23287960 DOI: 10.1097/IGC.0b013e31827b8430]
68 **James RE**, Lukanova A, Dossus L, Becker S, Rinaldi S, Tjønneland A, Olsen A, Overvad K, Mesrine S, Engel P, Clavel-Chapelon F, Chang-Claude J, Vrieling A, Boeing H, Schütze M, Trichopoulou A, Lagiou P, Trichopoulos D, Palli D, Krogh V, Panico S, Tumino R, Sacerdote C, Rodríguez L, Buckland G, Sánchez MJ, Amiano P, Ardanaz E, Bueno-de-Mesquita B, Ros MM, van Gils CH, Peeters PH, Khaw KT, Wareham N, Key TJ, Allen NE, Romieu I, Siddiq A, Cox D, Riboli E, Kaaks R. Postmenopausal serum sex steroids and risk of hormone receptor-positive and -negative breast cancer: a nested case-control study. *Cancer Prev Res* (Phila) 2011; **4**: 1626-1635 [PMID: 21813404 DOI: 10.1158/1940-6207.CAPR-11-0090]
69 **Allen NE**, Key TJ, Dossus L, Rinaldi S, Cust A, Lukanova A, Peeters PH, Onland-Moret NC, Lahmann PH, Berrino F, Panico S, Larrañaga N, Pera G, Tormo MJ, Sánchez MJ, Ramón Quirós J, Ardanaz E, Tjønneland A, Olsen A, Chang-Claude J, Linseisen J, Schulz M, Boeing H, Lundin E, Palli D, Overvad K, Clavel-Chapelon F, Boutron-Ruault MC, Bingham S, Khaw KT, Bueno-de-Mesquita HB, Trichopoulou A, Trichopoulos D, Naska A, Tumino R, Riboli E, Kaaks R. Endogenous sex hormones and endometrial cancer risk in women in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Endocr Relat Cancer* 2008; **15**: 485-497 [PMID: 18509001 DOI: 10.1677/ERC-07-0064]
70 **Bao C**, Yang X, Xu W, Luo H, Xu Z, Su C, Qi X. Diabetes mellitus and incidence and mortality of kidney cancer: a meta-analysis. *J Diabetes Complications* 2013; **27**: 357-364 [PMID: 23433629 DOI: 10.1016/j.jdiacomp.2013.01.004]
71 **Chow WH**, Gridley G, Fraumeni JF, Järvholm B. Obesity, hypertension, and the risk of kidney cancer in men. *N Engl J Med* 2000; **343**: 1305-1311 [PMID: 11058675 DOI: 10.1056/NEJM200011023431804]
72 **Russo P**. End stage and chronic kidney disease: associations with renal cancer. *Front Oncol* 2012; **2**: 28 [PMID: 22649783 DOI: 10.3389/fonc.2012.00028]
73 **Zhu Z**, Zhang X, Shen Z, Zhong S, Wang X, Lu Y, Xu C. Diabetes mellitus and risk of bladder cancer: a meta-analysis of cohort studies. *PLoS One* 2013; **8**: e56662 [PMID: 23437204 DOI: 10.1371/journal.pone.0056662]
74 **El-Mosalamy H**, Salman TM, Ashmawey AM, Osama N. Role of chronic E. coli infection in the process of bladder cancer- an experimental study. *Infect Agent Cancer* 2012; **7**: 19 [PMID: 22873280 DOI: 10.1186/1750-9378-7-19]
75 **Chari ST**, Leibson CL, Rabe KG, Ransom J, de Andrade M, Petersen GM. Probability of pancreatic cancer following diabetes: a population-based study. *Gastroenterology* 2005; **129**: 504-511 [PMID: 16083707 DOI: 10.1053/j.gastro.2005.05.007]
76 **Ben Q**, Xu M, Ning X, Liu J, Hong S, Huang W, Zhang H, Li Z. Diabetes mellitus and risk of pancreatic cancer: A meta-analysis of cohort studies. *Eur J Cancer* 2011; **47**: 1928-1937 [PMID: 21458985 DOI: 10.1016/j.ejca.2011.03.003]
77 **Liu J**, Knezetic JA, Strömmer L, Permert J, Larsson J, Adrian TE. The intracellular mechanism of insulin resistance in pancreatic cancer patients. *J Clin Endocrinol Metab* 2000; **85**: 1232-1238 [PMID: 10720068 DOI: 10.1210/jc.85.3.1232]
78 **Zhang F**, Yang Y, Skrip L, Hu D, Wang Y, Wong C, Qiu J, Lei H. Diabetes mellitus and risk of prostate cancer: an updated meta-analysis based on 12 case-control and 25 cohort studies. *Acta Diabetol* 2012; **49** Suppl 1: S235-S246 [PMID: 23124624 DOI: 10.1007/s00592-012-0439-5]
79 **Xu H**, Jiang HW, Ding GX, Zhang H, Zhang LM, Mao SH, Ding Q. Diabetes mellitus and prostate cancer risk of different grade or stage: a systematic review and meta-analysis. *Diabetes Res Clin Pract* 2013; **99**: 241-249 [PMID: 23298664 DOI: 10.1016/j.diabres.2012.12.003]
80 **Corona G**, Monami M, Rastrelli G, Aversa A, Sforza A, Lenzi A, Forti G, Mannucci E, Maggi M. Type 2 diabetes mellitus and testosterone: a meta-analysis study. *Int J Androl* 2011; **34**: 528-540 [PMID: 20969599 DOI: 10.1111/j.1365-2605.2010.01117.x]
81 **Yoon JM**, Son KY, Eom CS, Durrance D, Park SM. Pre-existing diabetes mellitus increases the risk of gastric cancer: a meta-analysis. *World J Gastroenterol* 2013; **19**: 936-945 [PMID: 23429469 DOI: 10.3748/wjg.v19.i6.936]
82 **Warren RS**, Yuan H, Matli MR, Ferrara N, Donner DB. Induction of vascular endothelial growth factor by insulin-like growth factor 1 in colorectal carcinoma. *J Biol Chem* 1996; **271**: 29483-29488 [PMID: 8910616 DOI: 10.1074/jbc.271.46.29483]
83 **Akagi Y**, Liu W, Zebrowski B, Xie K, Ellis LM. Regulation of vascular endothelial growth factor expression in human colon cancer by insulin-like growth factor-I. *Cancer Res* 1998; **58**: 4008-4014 [PMID: 9731515]
84 **Ikeda F**, Doi Y, Yonemoto K, Ninomiya T, Kubo M, Shikata K, Hata J, Tanizaki Y, Matsumoto T, Iida M, Kiyohara Y. Hyperglycemia increases risk of gastric cancer posed by Helicobacter pylori infection: a population-based cohort study. *Gastroenterology* 2009; **136**: 1234-1241 [PMID: 19236964 DOI:

10.1053/j.gastro.2008.12.045]
85 **Castillo JJ**, Mull N, Reagan JL, Nemr S, Mitri J. Increased incidence of non-Hodgkin lymphoma, leukemia, and myeloma in patients with diabetes mellitus type 2: a meta-analysis of observational studies. *Blood* 2012; **119**: 4845-4850 [PMID: 22496152 DOI: 10.1182/blood-2011-06-362830]
86 **Mitri J**, Castillo J, Pittas AG. Diabetes and risk of Non-Hodgkin's lymphoma: a meta-analysis of observational studies. *Diabetes Care* 2008; **31**: 2391-2397 [PMID: 19033419 DOI: 10.2337/dc08-1034]
87 **Barone BB**, Yeh HC, Snyder CF, Peairs KS, Stein KB, Derr RL, Wolff AC, Brancati FL. Long-term all-cause mortality in cancer patients with preexisting diabetes mellitus: a systematic review and meta-analysis. *JAMA* 2008; **300**: 2754-2764 [PMID: 19088353 DOI: 10.1001/jama.2008.824]
88 **Tian T**, Zhang LQ, Ma XH, Zhou JN, Shen J. Diabetes mellitus and incidence and mortality of gastric cancer: a meta-analysis. *Exp Clin Endocrinol Diabetes* 2012; **120**: 217-223 [PMID: 22187293 DOI: 10.1055/s-0031-1297969]
89 **Lin SY**, Hsieh MS, Chen LS, Chiu YH, Yen AM, Chen TH. Diabetes mellitus associated with the occurrence and prognosis of non-Hodgkin's lymphoma. *Eur J Cancer Prev* 2007; **16**: 471-478 [PMID: 17923820 DOI: 10.1097/01.cej.0000236253.93984.8f]
90 **Seshasai SR**, Kaptoge S, Thompson A, Di Angelantonio E, Gao P, Sarwar N, Whincup PH, Mukamal KJ, Gillum RF, Holme I, Njølstad I, Fletcher A, Nilsson P, Lewington S, Collins R, Gudnason V, Thompson SG, Sattar N, Selvin E, Hu FB, Danesh J. Diabetes mellitus, fasting glucose, and risk of cause-specific death. *N Engl J Med* 2011; **364**: 829-841 [PMID: 21366474 DOI: 10.1056/NEJMoa1008862]
91 **Barone BB**, Yeh HC, Snyder CF, Peairs KS, Stein KB, Derr RL, Wolff AC, Brancati FL. Postoperative mortality in cancer patients with preexisting diabetes: systematic review and meta-analysis. *Diabetes Care* 2010; **33**: 931-939 [PMID: 20351229 DOI: 10.2337/dc09-1721]
92 **Onitilo AA**, Engel JM, Glurich I, Stankowski RV, Williams GM, Doi SA. Diabetes and cancer II: role of diabetes medications and influence of shared risk factors. *Cancer Causes Control* 2012; **23**: 991-1008 [PMID: 22527174 DOI: 10.1007/s10552-012-9971-4]
93 **Norwood P**, Liutkus JF, Haber H, Pintilei E, Boardman MK, Trautmann ME. Safety of exenatide once weekly in patients with type 2 diabetes mellitus treated with a thiazolidinedione alone or in combination with metformin for 2 years. *Clin Ther* 2012; **34**: 2082-2090 [PMID: 23031623 DOI: 10.1016/j.clinthera.2012.09.007]
94 **Stein SA**, Lamos EM, Davis SN. A review of the efficacy and safety of oral antidiabetic drugs. *Expert Opin Drug Saf* 2013; **12**: 153-175 [PMID: 23241069 DOI: 10.1517/14740338.2013.752813]
95 **Hemmingsen B**, Christensen LL, Wetterslev J, Vaag A, Gluud C, Lund SS, Almdal T. Comparison of metformin and insulin versus insulin alone for type 2 diabetes: systematic review of randomised clinical trials with meta-analyses and trial sequential analyses. *BMJ* 2012; **344**: e1771 [PMID: 22517929 DOI: 10.1136/bmj.e1771]
96 **Soranna D**, Scotti L, Zambon A, Bosetti C, Grassi G, Catapano A, La Vecchia C, Mancia G, Corrao G. Cancer risk associated with use of metformin and sulfonylurea in type 2 diabetes: a meta-analysis. *Oncologist* 2012; **17**: 813-822 [PMID: 22643536 DOI: 10.1634/theoncologist.2011-0462]
97 **Rattan R**, Ali Fehmi R, Munkarah A. Metformin: an emerging new therapeutic option for targeting cancer stem cells and metastasis. *J Oncol* 2012; **2012**: 928127 [PMID: 22701483 DOI: 10.1155/2012/928127]
98 **Hirsch HA**, Iliopoulos D, Tsichlis PN, Struhl K. Metformin selectively targets cancer stem cells, and acts together with chemotherapy to block tumor growth and prolong remission. *Cancer Res* 2009; **69**: 7507-7511 [PMID: 19752085 DOI: 10.1158/0008-5472.CAN-09-2994]
99 **Hwang AL**, Haynes K, Hwang WT, Yang YX. Metformin and survival in pancreatic cancer: a retrospective cohort study. *Pancreas* 2013; **42**: 1054-1059 [PMID: 24051965 DOI: 10.1097/MPA.0b013e3182965a3c]
100 **Currie CJ**, Poole CD, Gale EA. The influence of glucose-lowering therapies on cancer risk in type 2 diabetes. *Diabetologia* 2009; **52**: 1766-1777 [PMID: 19572116 DOI: 10.1007/s00125-009-1440-6]
101 **Hsieh MC**, Lee TC, Cheng SM, Tu ST, Yen MH, Tseng CH. The influence of type 2 diabetes and glucose-lowering therapies on cancer risk in the Taiwanese. *Exp Diabetes Res* 2012; **2012**: 413782 [PMID: 22719752 DOI: 10.1155/2012/413782]
102 **Colmers IN**, Bowker SL, Majumdar SR, Johnson JA. Use of thiazolidinediones and the risk of bladder cancer among people with type 2 diabetes: a meta-analysis. *CMAJ* 2012; **184**: E675-E683 [PMID: 22761478 DOI: 10.1503/cmaj.112102]

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Diabetes, sleep apnea, obesity and cardiovascular disease: Why not address them together?

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Abstract

Obesity, sleep apnea, diabetes and cardiovascular diseases are some of the most common diseases encountered by the worldwide population, with high social and economic burdens. Significant emphasis has been placed on obtaining blood pressure, body mass index, and placing importance on screening for signs and symptoms pointing towards cardiovascular disease. Symptoms related to sleep, or screening for sleep apnea has been overlooked by cardiac, diabetic, pulmonary and general medicine clinics despite recommendations for screening by several societies. In recent years, there is mounting data where obesity and obstructive sleep apnea sit at the epicenter and its control can lead to improvement and prevention of diabetes and cardiovascular complications. This editorial raises questions as to why obstructive sleep apnea screening should be included as yet another vital sign during patient initial inpatient or outpatient visit.

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Key words: Obstructive sleep apnea; Diabetes; Obstructive sleep apnea screening; Obstructive sleep apnea; Cardiovascular complications

Core tip: Obesity, diabetes, cardiovascular disease and obstructive sleep apnea are one of the most common chronic diseases involving population globally. Efforts have been directed towards prevention and public education about the disease process of each of this condition separately. Though these diseases are interlinked, but educational efforts are failing short to address them together.

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OBSTRUCTIVE SLEEP APNEA

Should obstructive sleep apnea (OSA) screening be included as yet another vital sign during the patient first visit? Obesity and metabolic syndromes are emerging as major public health issues. One point one billion adults population worldwide are overweight, and approximately 312 million of them are obese[1]. Obesity is highly prevalent in United States but the prevalence is increasing in China, Southeast Asia, Middle East and Pacific Island[2]. The increasing incidence of childhood obesity and its association with the cardiovascular disease is also becoming a major public health concern[3,4]. The number of individuals inflicted with diabetes worldwide is approximately 285 million, but is expected to increase to 439 million by 2030[5]. 17 million deaths out of 57 million total worldwide deaths are attributable to cardiovascular disease[6]. The prevalence of OSA is between 4%-7% and increasing[7].

Obesity and OSA seem to be an epicenter for most of the chronic disease catastrophe. OSA is one of the most common diseases, with a high incidence and preva-

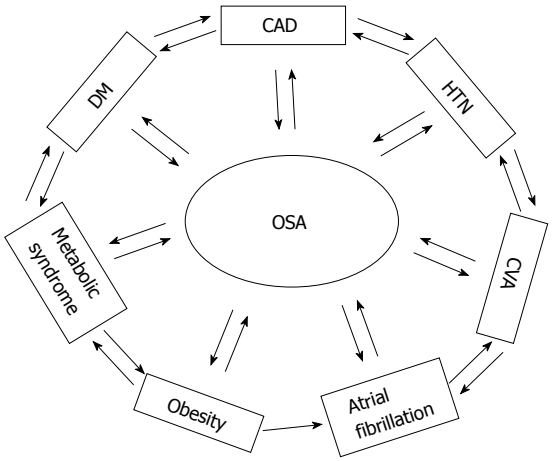

Figure 1 Showing the relationship of obstructive sleep apnea to cardiovascular diseases, diabetes, metabolic syndrome and obesity. CAD: Coronary artery disease; HTN: Hypertension; CVA: Cerebrovascular accident; DM: Diabetes mellitus; OSA: Obstructive sleep apnea.

lence rate that parallels with increasing obesity globally. OSA can be seen in non-obese patients with craniofacial abnormality and children with enlarged tonsils and adenoids too[8-10]. The growing prevalence of obesity and the increasing population body mass index has created major public health challenges[11]. Obstructive sleep apnea has been independently linked with hypertension, atrial fibrillation, cardiac disease, worsening of diabetes, insulin resistance, peri-operative and postoperative complications and coronary artery disease (CAD), to name the few[12-16]. In other words, the data links obstructive sleep apnea to a majority of chronic illnesses. In addition to the illness, untreated OSA increases the health care utilization, impairs work place efficiency, occupational injuries and increase healthcare utilization leading to billions of dollars in economic burden worldwide[17]. OSA if recognized can be adequately treated by an armamentarium of several different treatment modalities. Despite that 85% of the patients with clinically significant and treatable OSA have never been diagnosed, in other word the data has not made to the bedside[18].

OSA involves partial or complete collapse of the upper airway, despite respiratory efforts alternating with normal breathing. It affects 4%-7% of the population[7] and its prevalence in patients with cardiovascular disease is very high. Apnea is defined as a decline in peak signal excursion by $\geqslant$ 90% of their pre-event baseline for $\geqslant$ 10 s. Hypopnea is defined as a drop in the signal excursion by $\geqslant$ 30% of their pre-event baseline for $\geqslant$ 10% and $\geqslant$ 3% arterial oxygen desaturation or accompanied by an arousal[19]. OSA severity is based on Apnea-hypopnea index/h (AHI/h) It can be divided into mild OSA (AHI 5-15/h), Moderate OSA (AHI 15-30/h), and severe OSA (AHI > 30/h). The pathophysiology of obesity and OSA is intimately linked together. Obesity is a major risk factor for OSA. In obese patients there is an enlargement of soft tissue structures in the upper airway, leading to airway obstruction, especially during rapid eye movement sleep when there is atonia. In addition to obesity, there is an increase in fat deposition under the mandible, macroglossia, and palate, which can then lead to narrowing of airway and lead to apnea and hypopnea[20,21]. Obesity has been linked as the central and reversible cardiovascular risk factor that positively influences OSA, diabetes mellitus (DM), metabolic syndrome, hypertension, and lipid metabolism[17]. Children are not immune to the obesity, as the prevalence of obesity among children aged 2-5 is 10% and 6-19 years old is 15%[22].

OSA affects an estimated 15 million adult Americans, especially patients with hypertension, Atrial fibrillation (A-Fib), CAD, and congestive heart failure (CHF) where it is pervasive and levels are very high[23]. Additionally, OSA treatment has also been shown to improve atrial fibrillation incidence, coronary stent reclogging, and improvement of CHF and improvement in blood glucose and insulin resistance[24-29]. Recent evidence directly links OSA and obesity to CAD, heart failure, cardiomyopathy, A-Fib and DM and they are interrelated too as shown in Figure 1. The rise of obesity and DM has been an increased threat to the health of the global population, which has been catalyzed and compounded by the increased occurrence of OSA. In a recent study by Sleep AHEAD Research Group, OSA (AHI $\geqslant$ 5) was found to be in 86% of the population, whereas the pervasiveness of all forms of cardiovascular disease was 14%[30]. On the other hand, individuals who have DM and metabolic syndrome have an increased risk of cardiovascular disease and stroke[31].

The screening for OSA for commercial drivers has been suggested by several societies as American College of Chest Physician, American College of Occupational and Environmental Medicine, and National Sleep Foundation. The International Diabetes federation also recommends screening patients for possible OSA[32]. This screening among the commercial drivers has been successfully implemented, on the other hand, peri-operative screening has been suggested but not implemented in majority of the hospitals despite the availability of simple screening tools as STOP-Bang Questionnaire[33], Berlin Questionnaire[34], neck size, airway, morbidity, Epworth Sleepiness Score, snoring (NAMES) criteria, all with the sensitivity ranging from 80% to 86%[35].

This data has been in literature now for several years, indicating the associations of OSA with almost any disease as glaucoma, end stage renal disease, chronic obstructive pulmonary disease, polycystic ovarian syndrome, metabolic syndrome, cardiovascular disease, stroke, depression, obesity and DM. Moreover, the treatment has led to improvements in the underlying condition[36-38]. The screening test carries high sensitivity, but also has a low specificity. This can result in a plethora of false positive diagnosis and may increase the health care cost. There is high relationship between OSA, hypertension, cerebrovascular disease, CAD and A-Fib. Early diagnosis and treatment of OSA will help in preventing the increase morbidity and mortality associated with those conditions. Studies have shown the improvement in ejection fraction, carotid intimal thickening and benefits in

coronary artery disease, maintenance of sinus rhythm from A-Fib after cardioversion and improvement in insulin resistance. Moreover untreated OSA is also associated with increased risk of death[39-46]. The question arises, if it is the prime time to push for OSA screening for every patient walking in outpatient clinic or hospital? Or do we have to adjust the cutoff of points of our screening test so we can compromise with a decrease in sensitivity to have better specificity to avoid excess healthcare cost as a result of high false positive tests. It is the opinion of the author that Stop-Bang questionnaire, Berlin or NAMES questionnaire can be utilized as the screening tool. In the presence of symptoms, patient should undergo formal sleep study with home sleep study or overnight in lab polysomnography[33-35]. Regardless, one thing is clear: that every physician, nurse and midlevel provider needs to educate patients on risk prevention and education regarding the causes, signs and symptoms of diabetes, sleep apnea, obesity prevention and cardiovascular disease prevention. It is about time that health care providers take the responsibility of preventative education of such diseases as a package rather than fragmentation of education of diabetes in diabetic clinics, sleep apnea in sleep clinics, and cardiovascular disease in heart clinics, as these diseases are interrelated. I will leave the debate open as to if it is about time to push for screening of OSA as one of the vital signs on every patient initial visit.

REFERENCES

1 **Hossain P**, Kawar B, El Nahas M. Obesity and diabetes in the developing world--a growing challenge. *N Engl J Med* 2007; **356**: 213-215 [PMID: 17229948 DOI: 10.1056/NEJMp068177]

2 **Haidar YM**, Cosman BC. Obesity epidemiology. *Clin Colon Rectal Surg* 2011; **24**: 205-210 [PMID: 23204935 DOI: 10.1055/s-0031-1295684]

3 **Nguyen JV**, Robbins JM, Houck KL, Nobis EA, Inman KA, Khan KS, Robbins SW. Severe obesity and high blood pressure among children, Philadelphia health centers, 2010. *J Prim Care Community Health* 2014; **5**: 152-155 [PMID: 24327594]

4 **Luca AC**, Iordache C. Obesity--a risk factor for cardiovascular diseases. *Rev Med Chir Soc Med Nat Iasi* 2013; **117**: 65-71 [PMID: 24505894]

5 **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]

6 **Bovet PPF**. Cardiovascular disease and the changing face of global public health: a focus on low and middle income countries. *Public Health Review* 2012; **33**: 397-415

7 **Punjabi NM**. The epidemiology of adult obstructive sleep apnea. *Proc Am Thorac Soc* 2008; **5**: 136-143 [PMID: 18250205 DOI: 10.1513/pats.200709-155MG]

8 **Willington AJ**, Ramsden JD. Adenotonsillectomy for the management of obstructive sleep apnea in children with congenital craniosynostosis syndromes. *J Craniofac Surg* 2012; **23**: 1020-1022 [PMID: 22777462 DOI: 10.1097/SCS.0b013e31824e6cf8]

9 **Sutherland K**, Lee RW, Cistulli PA. Obesity and craniofacial structure as risk factors for obstructive sleep apnoea: impact of ethnicity. *Respirology* 2012; **17**: 213-222 [PMID: 21992683 DOI: 10.1111/j.1440-1843.2011.02082.x]

10 **Aihara K**, Oga T, Harada Y, Chihara Y, Handa T, Tanizawa K, Watanabe K, Hitomi T, Tsuboi T, Mishima M, Chin K. Analysis of anatomical and functional determinants of obstructive sleep apnea. *Sleep Breath* 2012; **16**: 473-481 [PMID: 21573913 DOI: 10.1007/s11325-011-0528-7]

11 **Zalesin KC**, Franklin BA, Miller WM, Peterson ED, McCullough PA. Impact of obesity on cardiovascular disease. *Med Clin North Am* 2011; **95**: 919-937 [PMID: 21855700 DOI: 10.1016/j.mcna.2011.06.005]

12 **Goyal SK**, Sharma A. Atrial fibrillation in obstructive sleep apnea. *World J Cardiol* 2013; **5**: 157-163 [PMID: 23802045 DOI: 10.4330/wjc.v5.i6.157]

13 **Bonsignore MR**, Borel AL, Machan E, Grunstein R. Sleep apnoea and metabolic dysfunction. *Eur Respir Rev* 2013; **22**: 353-364 [PMID: 23997062 DOI: 10.1183/09059180.00003413]

14 **Hamilton GS**, Naughton MT. Impact of obstructive sleep apnoea on diabetes and cardiovascular disease. *Med J Aust* 2013; **199**: S27-S30 [PMID: 24138362 DOI: 10.5694/mja13.10579]

15 **Tahrani AA**, Ali A, Stevens MJ. Obstructive sleep apnoea and diabetes: an update. *Curr Opin Pulm Med* 2013; **19**: 631-638 [PMID: 24048079 DOI: 10.1097/MCP.0b013e3283659da5]

16 **Nepomnayshy D**, Hesham W, Erickson B, MacDonald J, Iorio R, Brams D. Sleep apnea: is routine preoperative screening necessary? *Obes Surg* 2013; **23**: 287-291 [PMID: 23104390 DOI: 10.1007/s11695-012-0806-x]

17 **AlGhanim N**, Comondore VR, Fleetham J, Marra CA, Ayas NT. The economic impact of obstructive sleep apnea. *Lung* 2008; **186**: 7-12 [PMID: 18066623 DOI: 10.1007/s00408-007-9055-5]

18 **Somers VK**, White DP, Amin R, Abraham WT, Costa F, Culebras A, Daniels S, Floras JS, Hunt CE, Olson LJ, Pickering TG, Russell R, Woo M, Young T. Sleep apnea and cardiovascular disease: an American Heart Association/american College Of Cardiology Foundation Scientific Statement from the American Heart Association Council for High Blood Pressure Research Professional Education Committee, Council on Clinical Cardiology, Stroke Council, and Council On Cardiovascular Nursing. In collaboration with the National Heart, Lung, and Blood Institute National Center on Sleep Disorders Research (National Institutes of Health). *Circulation* 2008; **118**: 1080-1111 [PMID: 18725495 DOI: 10.1161/CIRCULATIONAHA.107.189420]

19 **Berry RB**, Budhiraja R, Gottlieb DJ, Gozal D, Iber C, Kapur VK, Marcus CL, Mehra R, Parthasarathy S, Quan SF, Redline S, Strohl KP, Davidson Ward SL, Tangredi MM. Rules for scoring respiratory events in sleep: update of the 2007 AASM Manual for the Scoring of Sleep and Associated Events. Deliberations of the Sleep Apnea Definitions Task Force of the American Academy of Sleep Medicine. *J Clin Sleep Med* 2012; **8**: 597-619 [PMID: 23066376]

20 **Schwab RJ**, Pasirstein M, Pierson R, Mackley A, Hachadoorian R, Arens R, Maislin G, Pack AI. Identification of upper airway anatomic risk factors for obstructive sleep apnea with volumetric magnetic resonance imaging. *Am J Respir Crit Care Med* 2003; **168**: 522-530 [PMID: 12746251 DOI: 10.1164/rccm.200208-866OC]

21 **Horner RL**, Shea SA, McIvor J, Guz A. Pharyngeal size and shape during wakefulness and sleep in patients with obstructive sleep apnoea. *Q J Med* 1989; **72**: 719-735 [PMID: 2602554]

22 **Smith I**, Lasserson TJ. Pressure modification for improving usage of continuous positive airway pressure machines in adults with obstructive sleep apnoea. *Cochrane Database Syst Rev* 2009; (**4**): CD003531 [PMID: 19821310 DOI: 10.1002/14651858]

23 **Somers VK**, White DP, Amin R, Abraham WT, Costa F, Culebras A, Daniels S, Floras JS, Hunt CE, Olson LJ, Pickering TG, Russell R, Woo M, Young T. Sleep apnea and cardiovascular disease: an American Heart Association/American College of Cardiology Foundation Scientific Statement from the American Heart Association Council for High Blood

Pressure Research Professional Education Committee, Council on Clinical Cardiology, Stroke Council, and Council on Cardiovascular Nursing. *J Am Coll Cardiol* 2008; **52**: 686-717 [PMID: 18702977 DOI: 10.1016/j.jacc.2008.05.002]

24 **Diaz-Melean CM**, Somers VK, Rodriguez-Escudero JP, Singh P, Sochor O, Llano EM, Lopez-Jimenez F. Mechanisms of adverse cardiometabolic consequences of obesity. *Curr Atheroscler Rep* 2013; **15**: 364 [PMID: 24048571 DOI: 10.1007/s11883-013-0364-2]

25 **Mansukhani MP**, Calvin AD, Kolla BP, Brown RD, Lipford MC, Somers VK, Caples SM. The association between atrial fibrillation and stroke in patients with obstructive sleep apnea: a population-based case-control study. *Sleep Med* 2013; **14**: 243-246 [PMID: 23340087 DOI: 10.1016/j.sleep.2012.08.021]

26 **Kourouklis SP**, Vagiakis E, Paraskevaidis IA, Farmakis D, Kostikas K, Parissis JT, Katsivas A, Kremastinos DT, Anastasiou-Nana M, Filippatos G. Effective sleep apnoea treatment improves cardiac function in patients with chronic heart failure. *Int J Cardiol* 2013; **168**: 157-162 [PMID: 23041002 DOI: 10.1016/j.ijcard.2012.09.101]

27 **Bopparaju S**, Surani S. Sleep and diabetes. *Int J Endocrinol* 2010; **2010**: 759509 [DOI: 10.1155/2010/759509]

28 **Surani S**, Subramanian S. Effect of continuous positive airway pressure therapy on glucose control. *World J Diabetes* 2012; **3**: 65-70 [PMID: 22532885 DOI: 10.4239/wjd.v3.i4.65]

29 **Surani S**. Are diabetic patients being screened for sleep related breathing disorder? *World J Diabetes* 2013; **4**: 162-164 [PMID: 24147199]

30 **Rice TB**, Foster GD, Sanders MH, Unruh M, Reboussin D, Kuna ST, Millman R, Zammit G, Wing RR, Wadden TA, Kelley D, Pi-Sunyer X, Newman AB. The relationship between obstructive sleep apnea and self-reported stroke or coronary heart disease in overweight and obese adults with type 2 diabetes mellitus. *Sleep* 2012; **35**: 1293-1298 [PMID: 22942508]

31 **Ramar K**, Surani S. The relationship between sleep disorders and stroke. *Postgrad Med* 2010; **122**: 145-153 [PMID: 21084791 DOI: 10.3810/pgm.2010.11.2232]

32 **Shaw JE**, Punjabi NM, Wilding JP, Alberti KG, Zimmet PZ. Sleep-disordered breathing and type 2 diabetes: a report from the International Diabetes Federation Taskforce on Epidemiology and Prevention. *Diabetes Res Clin Pract* 2008; **81**: 2-12 [PMID: 18544448 DOI: 10.1016/j.diabres.2008.04.025]

33 **Chung F**, Yegneswaran B, Liao P, Chung SA, Vairavanathan S, Islam S, Khajehdehi A, Shapiro CM. STOP questionnaire: a tool to screen patients for obstructive sleep apnea. *Anesthesiology* 2008; **108**: 812-821 [PMID: 18431116 DOI: 10.1097/ALN.0b013e31816d83e4]

34 **Netzer NC**, Stoohs RA, Netzer CM, Clark K, Strohl KP. Using the Berlin Questionnaire to identify patients at risk for the sleep apnea syndrome. *Ann Intern Med* 1999; **131**: 485-491 [PMID: 10507956 DOI: 10.7326/0003-4819-131-7-199910050-00002]

35 **Subramanian S**, Hesselbacher SE, Aguilar R, Surani SR. The NAMES assessment: a novel combined-modality screening tool for obstructive sleep apnea. *Sleep Breath* 2011; **15**: 819-826 [PMID: 21076972 DOI: 10.1007/s11325-010-0443-3]

36 **Subramanian S**, Guntupalli B, Murugan T, Bopparaju S, Chanamolu S, Casturi L, Surani S. Gender and ethnic differences in prevalence of self-reported insomnia among patients with obstructive sleep apnea. *Sleep Breath* 2011; **15**: 711-715 [PMID: 20953842 DOI: 10.1007/s11325-010-0426-4]

37 **Subramanian S**, Desai A, Joshipura M, Surani S. Practice patterns of screening for sleep apnea in physicians treating PCOS patients. *Sleep Breath* 2007; **11**: 233-237 [PMID: 17541663 DOI: 10.1007/s11325-007-0120-3]

38 **Lee R**, McNicholas WT. Obstructive sleep apnea in chronic obstructive pulmonary disease patients. *Curr Opin Pulm Med* 2011; **17**: 79-83 [PMID: 21169840 DOI: 10.1097/MCP.0b013e32834317bb]

39 **Drager LF**, Bortolotto LA, Lorenzi MC, Figueiredo AC, Krieger EM, Lorenzi-Filho G. Early signs of atherosclerosis in obstructive sleep apnea. *Am J Respir Crit Care Med* 2005; **172**: 613-618 [PMID: 15901608 DOI: 10.1164/rccm.200503-340OC]

40 **Drager LF**, Bortolotto LA, Figueiredo AC, Krieger EM, Lorenzi GF. Effects of continuous positive airway pressure on early signs of atherosclerosis in obstructive sleep apnea. *Am J Respir Crit Care Med* 2007; **176**: 706-712 [PMID: 17556718 DOI: 10.1164/rccm.200703-500OC]

41 **Yaggi HK**, Concato J, Kernan WN, Lichtman JH, Brass LM, Mohsenin V. Obstructive sleep apnea as a risk factor for stroke and death. *N Engl J Med* 2005; **353**: 2034-2041 [PMID: 16282178 DOI: 10.1056/NEJMoa043104[published]]

42 **Young T**, Finn L, Peppard PE, Szklo-Coxe M, Austin D, Nieto FJ, Stubbs R, Hla KM. Sleep disordered breathing and mortality: eighteen-year follow-up of the Wisconsin sleep cohort. *Sleep* 2008; **31**: 1071-1078 [PMID: 18714778]

43 **Mooe T**, Rabben T, Wiklund U, Franklin KA, Eriksson P. Sleep-disordered breathing in men with coronary artery disease. *Chest* 1996; **109**: 659-663 [PMID: 8617073 DOI: 10.1378/chest.109.3.659]

44 **Ip MS**, Tse HF, Lam B, Tsang KW, Lam WK. Endothelial function in obstructive sleep apnea and response to treatment. *Am J Respir Crit Care Med* 2004; **169**: 348-353 [PMID: 14551167 DOI: 10.1164/rccm.200306-767OC]

45 **Gami AS**, Pressman G, Caples SM, Kanagala R, Gard JJ, Davison DE, Malouf JF, Ammash NM, Friedman PA, Somers VK. Association of atrial fibrillation and obstructive sleep apnea. *Circulation* 2004; **110**: 364-367 [PMID: 15249509 DOI: 10.1161/01.CIR.0000136587.68725.8E]

46 **Cassar A**, Morgenthaler TI, Lennon RJ, Rihal CS, Lerman A. Treatment of obstructive sleep apnea is associated with decreased cardiac death after percutaneous coronary intervention. *J Am Coll Cardiol* 2007; **50**: 1310-1314 [PMID: 17903628 DOI: 10.1016/j.jacc.2007.06.028]

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MINIREVIEWS

Emerging role of protein kinase C in energy homeostasis: A brief overview

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Abstract

Protein kinase C-β (PKCβ), a member of the lipid-activated serine/threonine PKC family, has been implicated in a wide range of important cellular processes. Very recently, the novel role of PKCβ in the regulation of triglyceride homeostasis *via* regulating mitochondrial function has been explored. In this review, I aim to provide an overview of PKCβ regarding regulation by lipids and recently gained knowledge on its role in energy homeostasis. Alterations in adipose PKCβ expression have been shown to be crucial for diet-induced obesity and related metabolic abnormalities. High-fat diet is shown to induce PKCβ expression in white adipose tissue in an isoform- and tissue-specific manner. Genetically manipulated mice devoid of PKCβ are lean with increased oxygen consumption and are resistant to high-fat diet-induced obesity and hepatic steatosis with improved insulin sensitivity. Available data support the model in which PKCβ functions as a "diet-sensitive" metabolic sensor whose induction in adipose tissue by high-fat diet is among the initiating event disrupting mitochondrial homeostasis *via* intersecting with $p66^{Shc}$ signaling to amplify adipose dysfunction and have systemic consequences. Alterations in PKCβ expression and/or function may have important implications in health and disease and warrants a detailed investigation into the downstream target genes and the underlying mechanisms involved. Development of drugs that target the PKCβ pathway and identification of miRs specifically controlling PKCβ expression may lead to novel therapeutic options for treating age-related metabolic disease including fatty liver, obesity and type 2 diabetes.

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Key words: High-fat diet; Signal transduction; Obesity; Mitochondrial function; Insulin resistance

Core tip: Nutrition has important long-term consequences for health. It is one of the lifestyle factors that contribute to the development and progression of obesity (increased fat accumulation), diabetes, and cardiovascular diseases. In fact, obesity rates are increasing dramatically worldwide and obesity amplifies the risk of developing various age-related chronic diseases, such as type 2 diabetes and cardiovascular disease. The prevention or management of chronic diseases is a global priority since they constitute a serious strain on health care systems and account for more than half of the deaths worldwide. Although correct lifestyle remains the mainstream solution to this problem, pharmacological strategies are also being actively seeked. Current antiobesity strategies have not controlled increasing epidemic of obesity and obesity-related disorders. We hope that a better knowledge of the molecular players and biochemical mechanism linking dietary fat to fat accumulation and development of glucose intolerance are critically needed. This review examines a way of metabolizing dietary fat into heat instead of storing them as fat, and the possibility that the "browning" of white fat is regulated by a diet-inducible kinase Protein kinase C-β (PKCβ) may help us explore new translational approaches to combat obesity, improve insulin sensitivity and potentially increase longevity. Finally, attenuation of inflammation in fat by PKCβ inhibition

could have profound clinical consequences because of the large size of the fat organ and its central metabolic role.

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INTRODUCTION

Protein kinase C (PKC) family is the largest serine/threonine-specific kinase family known to comprise approximately 2% of the human kinome[1]. PKCs are broadly conserved in eukaryotes, ranging in complexity from a single isoform in budding yeast (Saccharomyces cerevisiae) to 5 isoforms in Drosophila melanogaste and 12 in mammals[2,3]. Three distinct subfamilies can be identified according to their dependency on three combinations of activators: conventional (α, βⅠ, βⅡ, γ) require phosphatidylserine, diacylglycerol, and Ca^{2+}; novel (δ, ε, η, θ) need phosphatidylserine (PS) and DAG but not Ca^{2+}; atypical PKCs (λ/ι, ζ) are insensitive to both DAG and Ca^{2+}. PKC isoforms differ in primary structure, tissue distribution, subcellular localization, *in vitro* mode of action, response to extracellular signals, and substrate specificity. The role of individual PKC isoform is thought to be determined through sub isoform-specific activation processes or isoform-specific substrates in the region downstream of the PKC pathway[4]. Specific role of each isoform is beginning to be understood using isoform-specific transgenic and knockout mouse models. PKCs have been extensively discussed in the literature, and the aim of this review is to focus on the functions of PKCβ in the context of obesity and related metabolic syndromes.

REGULATION OF PKCβ ACTIVITY AND EXPRESSION BY LIPIDS

PKCβ is unique among all PKC isoforms in that a single gene locus encodes two proteins, PKCβⅠ and PKCβⅡ, which are generated by alternative splicing of C-terminal exons and are shown to be physiologically relevant[5]. The difference between these two isoforms resides in the C-terminal V5 domains, which still exhibit a moderate homology (45%) at their amino acid sequences[6,7]. PKCβ is highly expressed in the brain and adipose tissue, and widely expressed at a lower level in multiple tissues including liver, kidney, and skeletal muscle. Analysis of the primary structure of PKCβ reveals the presence of four domains conserved across PKC isoforms (C1-C4) and five variable domains that are divergent (V1-V5). Two functional domains have been described: an amino terminal regulatory domain and a carboxyl terminal catalytic domain. The regulatory domain (V1-V3) contains the so-called pseudosubstrate site which is thought to interact

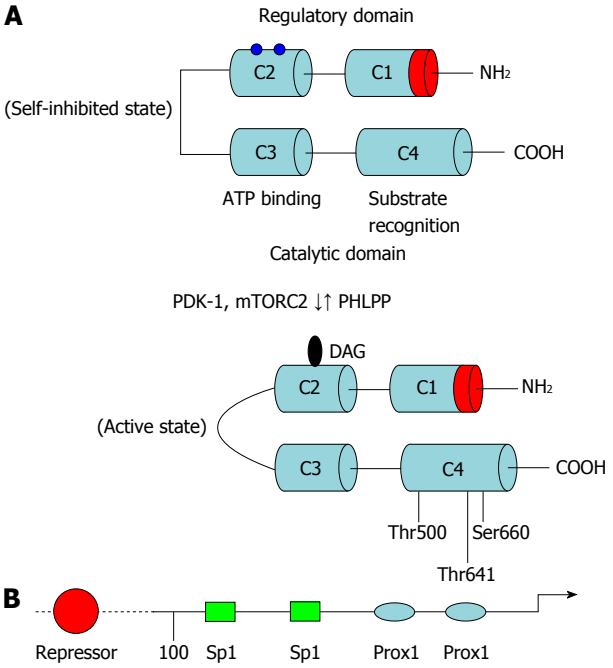

Figure 1 Domain composition of protein kinase C-β and its regulation at the transcriptional and posttranscriptional levels. A: Membrane-targeting modules (C1 and C2), pleckstrin homology domain, the pseudosubstrate region, the kinase core and the C-terminal tail; B: Schematic representation of promoter structure of protein kinase C-β gene. Approximate locations of known regulatory regions are indicated. ATP: Adenosine-5'-triphosphate; PHLPP: PH domain and leucine rich repeat protein phosphatases; PDK-1: 3-phosphoinositide-dependent protein kinase 1.

with the catalytic domain to retain PKCβ in an inactive conformation. The regulatory domain also contains sites for the interaction of PKC with PS, DAG/phorbol ester, and Ca^{2+}. The Ca^{2+} dependency is mediated by the C2 region, while phorbol-ester binding requires the presence of two cysteine-rich zinc finger regions within the C1 domain. The catalytic domain contains two conserved regions, C3 and C4, which are essential for the kinase activity and the binding of adenosine-5'-triphosphate (ATP)/substrate (Figure 1).

In addition to the above specific inputs, other regulatory processes influence the function of PKCβ, including phosphorylation and interaction with specific binding partners. PKCβ is processed by three distinct phosphorylation events before it is competent to respond to the coactivators and is phosphorylated at three conserved serine/threonine residues in the C-terminal domain[8]. Phosphorylation at the activation loop (Thr^{500}) is generally proposed to be first and to be followed by two ordered phosphorylations at the C-terminal tail, the turn motif (Thr^{641} in PKCβⅡ) and then the hydrophobic motif (Ser^{660} in PKCβⅡ). The phosphorylation of the turn motif depends on the mTORC2 complex; this phosphorylation triggers autophosphorylation of the hydrophobic motif[9,10]. The fully-phosphorylated "mature" PKCβ is in a closed conformation in which the pseudosubstrate occupies the substrate-binding cavity, thus autoinhibiting the kinase. Signals that cause hydrolysis of phosphatidylinositol-4,5-bisphosphate result in trans-

location of PKCβ to the membrane by a low-affinity interaction where it binds DAG *via* the C1 domain. Engaging both the C1 and C2 domains on the membrane results in a high-affinity membrane interaction that results in release of the pseudosubstrate, allowing downstream signaling. The membrane-bound conformation is highly phosphatase-sensitive, so that prolonged membrane binding results in dephosphorylation of PKCβ by pleckstrin homology domain Leucine-rich repeat protein phosphatase and PP2A, and subsequent degradation[11]. Binding of Hsp70 to the dephosphorylated turn motif on the C-terminus stabilizes PKCβ, allowing it to become rephosphorylated and reenter the pool of signaling-competent PKC. PKCβ that is not rescued by hsp70 is ubiquitinated by E3 ligases such as the recently discovered RINCK and degraded[12].

PKCβ is also responsive to oxidative stress[13-15]. Why is PKCβ sensitive to oxidative stress? In the PKCβ structure, two pairs of zinc fingers are found within the regulatory domain. They are sites of DAG and phorbol ester binding. Each zinc finger is formed by a structure that is composed of six cysteine residues and two zinc atoms. The high level of cysteine residues renders the regulatory domain susceptible to redox regulation[16,17]. The oxidant destroys the zinc finger conformation, and the autoinhibition is relieved, resulting in a PKCβ form that is catalytically active in the absence of Ca^{2+} or phospholipids[18].

Besides the lipid activation at the post-transcriptional level, PKCβ expression also fluctuates in response to high-fat diet intake. It is shown that feeding high-fat diet (HFD) for 12 wk induces adipose PKCβ expression in an isoform and tissue-specific manner[19]. The molecular mechanism(s) underlying transcription induction have yet to be elucidated but previous studies have cloned and sequenced PKCβ promoter[20-22]. A putative 5'-promoter region for PKCβ is identified and suggested that there is heterogeneity in the active promoter region dependent upon the cellular context. Analysis of the 5'-promoter of PRKCB revealed that a region between -110 bp and -48 bp contains two Sp1 binding sites which are important for basal expression of *PKCβ* gene. In addition two PROX1 sites are also present 3' to Sp1 sites and are involved in inhibiting Sp1-mediated basal transcription of PKCβ promoter[23]. In fact, an inverse relationship between PROX1 and PKCβ levels exist in colon cancer cell lines. It was also found that treatment with a demethylating agent, 5-aza-2'-deoxycytidine, restored PKCβ mRNA expression in PROX1-expressing cells, suggesting that the 5'-promoter of PKCβ is methylated in these cells[23]. Actually, a CpG island in this region, in particular a CpG site within the distal Sp1 site is identified in this study, leading to downregulation of PKCβ transcription. Hypermethylation of PROX1 sites inhibits direct Sp1 binding to this region in PROX1 overexpressing cells. Finally, previous studies have also identified a repressor region located upstream of -110 bp in the PKCβ promoter and the identity of the nuclear factor(s) binding to this region has not been characterized.

NOVEL ROLE OF PKCβ IN LIPID HOMEOSTASIS

A significant conceptual advance in our understanding of the importance of PKCβ signaling in obesity has come from realization that mice deficient in PKCβ express higher levels of genes that regulate fatty acid oxidation and proteins involved in energy dissipation, highlighting its role as a corepressor and in controlling the balance between energy consumption and energy expenditure[24]. On the contrary, genes involved in FA synthesis and gluconeogenesis seem to be downregulated in the absence of PKCβ[25,26]. As a consequence, PKCβ mice are lean, with a significant reduction of body fat and body weight compared to WT mice and are resistant to HFD-induced obesity and hepatic steatosis so that these mice maintain their insulin sensitivity[19]. Moreover, PKCβ levels are shown to be elevated in adipose tissue of leptin-deficient (ob/ob) mice and deletion of PKCβ in ob/ob mice attenuates obesity syndrome of these mice[26]. An important mechanistic insight is the revelation that in PKCβ-deficient mice white adipose tissue (WAT) express genes characteristic of BAT including peroxisome proliferator-activated receptor-gamma coactivator-1alpha (PGC-1α), fatty acid transporter carnitine palmitoyltransferase, and uncoupling protein-1 (UCP-1). Targeted disruption in mice of several genes directly involved in energy metabolism and fat accumulation also leads to lean phenotype with a marked increase in UCP-1 expression in adipocytes, particularly in white fat depots[27-29]. Thus total energy consumption is increased significantly in PKCβ-null mice, presumably as a consequence of energy dissipation in WAT resulting from the expression of UCP-1 and increased mitochondrial activity. The ability of white and brown adipocytes in each depot to reversibly switch into one another has been reported, but the extent to which this occurs and the precise mechanisms involved are not fully understood. The search for regulators that could mediate conversion of white adipocytes (energy storing) into brown adipocytes (energy consuming) has led to the identification of PGC-1α, FOXC2 and positive regulatory domain-containing 16 as transcriptional regulators that have been found to promote a brown fat genetic program, while retinoblastoma protein and RIP140 have been described to favor a white adipose phenotype[27-30]. Another important aspect of these studies relates to possible connection between PKCβ and β-adrenergic receptor levels in WAT. Results presented argue strongly in favor of an inverse relationship between PKCβ and β3-adrenergic receptor expression[26]. The proposed relationship is consistent with earlier reports showing that sustained PKC activation suppressed β-ARs expression at the transcriptional level[31-33]. The net consequence of PKCβ-mediated adipose dysfunction could have profound clinical consequences because of the large size of the fat organ and its central metabolic role. Interestingly, in agreement with the above animal studies, adipose

PKCβ activation is subsequently linked to obese side effects of antipsychotic drugs in humans[34]. Moreover, in agreement with its role in energy homeostasis, PKCβ is shown to be required for adipocyte differentiation[35], PKCβ inhibition promotes insulin signaling in adipocytes[36,37], and PKCβ promoter polymorphism is associated with insulin resistance in humans[38].

The role of PKCβ in obesity is further supported by its potential involvement in angiogenesis. To ensure a sufficient supply of nutrients and oxygen and to transport fatty acids and adipokines, an extended microvasculature is mandatory for adipose tissue. Adipogenesis and angiogenesis are two closely related processes during adipose tissue enlargement, as shown in animal studies and *in vitro* models[39,40]. As adipocyte hypertrophy endures, local adipose tissue hypoxia may occur due to hypoperfusion since the diameter of fat cells overgrows the diffusion limit of oxygen. As a result, hypoxia-inducible transcription factors are expressed triggering the expression of angiogenic factors [vasuclar endothelial growth factor (VEGF), hepatocyte growth factor, plasminogen activator inhibitor-1]. In view of role of PKCβ/HuR in regulating VEGF expression at the post-transcriptional level, simultaneous induction of PKCβ is expected to promote VEGF expression[41,42].

Finally, specific overexpression of a constitutively active PKCβ Ⅱ mutant in mouse skeletal muscle demonstrated that this splice variant of PKCβ not only induces insulin resistance, but also affects the levels of several genes involved in lipid metabolism[43]. Thus impairment in the expression of PGC-1α, acyl CoA oxidase and hormone-sensitive lipase, but enhanced expression of the lipogenic transcription factor sterol response element-binding protein 1c in skeletal muscle, were associated with decreased lipid oxidation and increased intra-myocellular lipid deposition. In addition to these direct effects in muscle, these animals showed defects in insulin action in the liver and brain, as well as hepatic lipid accumulation similar to that seen in fat-fed animals.

POTENTIAL ROLE OF PKCβ IN MITOCHONDRIAL FUNCTION

Several studies have emphasized the association between enhanced mitochondria-derived H_2O_2 and insulin resistance, particularly in the context of excessive nutrient intake that results in metabolic imbalance[44-47]. Oxidative stress has also been described clinically, as well as in WAT of many additional mouse models of obesity, such as the KKAy and db/db mice. Systemic markers of oxidative stress increase with adiposity, consistent with the role of reactive oxygen species (ROS) in the development of obesity-induced insulin resistance. Available data suggest that an increase in ROS significantly affects WAT biology and leads to deregulated expression of inflammatory cytokines such as tumor necrosis factor-α, interleukin-6, and macrophage chemoattractant protein-1, and insulin resistance, which could contribute to obesity-associated diabetes and cardiovascular diseases[47]. Moreover, oxidative stress induced by ROS stimulates fat tissue development both *in vitro* and *in vivo*. H_2O_2-induced oxidative stress is shown to facilitate the differentiation of preadipocytes into adipocytes by accelerating mitotic clonal expansion[48]. Antioxidants such as flavonoids and N-acetylcysteine inhibit both adipogenic transcription factors C/EBP-β and PPAR-γ expression, as well as adipogenic differentiation in 3T3-L1 preadipocytes[49,50]. N-acetyl cysteine (NAC) was also shown to reduce ROS levels and fat accumulation in a concentration-dependent manner[50]. Moreover, animals on a HFD with the antioxidant NAC exhibited lower visceral fat and body weight[51]. Finally, ROS scavenging is associated with fat reduction in obese Zucker rats[52].

Recent studies have highlighted a novel, unexpected signaling pathway bridging the oxidative challenge of a cell to the activation of PKCβ/$p66^{Shc}$-controlled mitochondrial lifespan[53,54]. PKCβ activated by oxidative stress is shown to be required for phosphorylation of the Ser36 of $p66^{Shc}$ and the effect of PKCβ overexpression on mitochondrial Ca^{2+} signaling was not observed in $p66^{Shc-/-}$ cells. Importantly, the mitochondrial consequences of hydrogen peroxide are blocked by hispidine, a specific PKCβ inhibitor. The pathway emerging from these studies is the following: during oxidative stress PKCβ is activated and induces $p66^{Shc}$ phosphorylation, thus allowing $p66^{Shc}$ to be recognized by Pin1, isomerised and imported into mitochondria after dephosphorylation by type 2 protein serine/threonine phosphatase. The $p66^{Shc}$ protein translocated into the appropriate cell domain, can exert the oxidoreductase activity, generating H_2O_2 and inducing the opening of MPTP. This event in turn perturbs mitochondria structure and function. Identification of a novel signaling mechanism, which is operative in the pathophysiological condition of oxidative stress, may open new possibilities for pharmacologically addressing the process of organ deterioration during aging. The above studies are among the first to dissect the downstream target genes and regulatory properties of the PKCβ protein, and therefore make an important contribution to our understanding of the molecular basis to the lean phenotype exhibited by $PKCβ^{-/-}$ mice. Based on a very recent demonstration that PKCβ/$p66^{Shc}$ mitochondrial axis inhibits autophagy[55] and the evolving role of autophagy in energy homeostasis[56-61], it is possible that a combination of adipose PKCβ activation, mitochondrial dysfunction and insufficient autophagy may contribute to the development of diet-induced obesity. In addition to mitochondrial effects, PKCβ is an upstream regulator of NOX but this signaling axis actively produces superoxide across the membranes of neutrophils and phagosomes[62-65]. Accumulating data so far implicates mitochondria as the main source for regulation of autophagy by ROS production in adipocytes[66], whereas NOX contributes to activation of selective, bacterial autophagy[67] (Figure 2).

Although biological function of PKCβ in energy

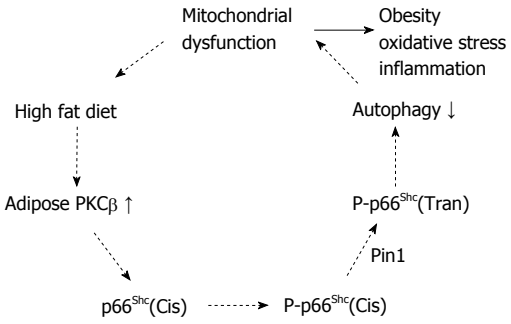

Figure 2 Proapoptotic signals, including reactive oxygen species, activate protein kinase C-β, which in turn phosphorylates p66^{Shc} at serine 36. Phosphorylated p66^{Shc} translocates to the inner mitochondrial membrane and acts as a redox enzyme to amplify oxidative stress by generating H_2O_2. Increased H_2O_2, in turn, causes opening of the mitochondrial permeability transition pore and apoptosis. Protein kinase C-β (PKCβ) activated by reactive oxygen species further induces p66^{Shc} phosphorylation. This event in turn perturbs mitochondria structure and function.

homeostasis appears to be mostly linked with events occurring at the mitochondria, however, increasing evidence has implied a role for this kinase in nuclear functions, suggesting this may be a pathway to communicate signals generated at the plasma membrane to the nucleus. For example, Goss *et al*[68] first showed that PKCβ translocates to the nucleus at G2/M, concomitant with the phosphorylation of lamin B1. Subsequently, a considerable number of nuclear proteins have been identified which are *in vivo* and/or *in vitro* substrates for PKCβ. These proteins include: histone H3, DNA topoisomerase I and Ⅱa, DNA polymerase α and β, cyclic AMP-response element-binding protein, retinoblastoma protein, and vitamin D receptor[69-73]. It has even been shown that PKCβ I co-localizes with androgen receptor and lysine-specific demethylase 1 on target gene promoters and phosphorylation of histone H3 at threonine 6 by PKCβ I is the key event that prevents lysine-specific demethylase 1 from demethylating histone H3 lysine 4[69]. Finally, activated PKCβ indirectly can affect other signaling cascades, including PI3-kinase/Akt pathway, extracellular signal-regulated kinase, and p38 pathway which can impact nuclear events[74-79]. It is thus clear that characterization of PKCβ downstream signaling in the nucleus and its relevance to energy homeostasis is another facets that requires in-depth investigation.

The above findings are applicable to the pathogenesis of obesity and type 2 diabetes since mitochondrial loss in WAT correlates with the development of obesity and type 2 diabetes[80,81]. Indeed, mitochondrial DNA copy number, mitochondrial mass, and mitochondrial activity are all decreased in the white adipose tissue of mouse models of obesity, such as ob/ob and db/db mice[82,83]. Similarly in patients with insulin resistance, type 2 diabetes, and severe obesity, the abundance of mitochondria and the expression of key genes pertinent to mitochondrial function are significantly reduced in white adipose tissue, in concert with decreased adipocyte oxygen consumption rates and ATP production[84,85]. The mitochondrial dysfunction, which could impair substrate oxidation in adipose tissue, is thought to participate in metabolic impairment capacity, thereby accentuating the development of obesity and associated pathologies, such as type 2 diabete. As a result, WAT mitochondria are emerging as highly attractive organelles for therapeutic interventions with the potential to impact upon systemic metabolism. Interestingly, the insulin-sensitizing effects of thiazolidinediones are closely matched by robust increases in adipose tissue mitochondrial biogenesis[86].

CONCLUSION

We have reviewed recent advances pertaining to the potential role of PKCβ in regulating energy homeostasis and contribution to the development of metabolic syndrome. Evidence gathered recently point to an essential role for PKCβ in diet-induced obesity. As a signaling pathway, PKCβ is highly sensitive to changes in environment and fluctuations in lipid supply activate adipose PKCβ, which in turn appears to promote fat accumulation *via* modulating mitochondrial function. A positive loop between oxidative stress and PKCβ/p66^{Shc} is promising and may be the major mechanism underlying contribution of PKCβ activation in generating oxidative stress observed in the obese state. The main gap in our understanding today lies in the specific, molecular and chemical mechanisms of PKCβ-mediated energy homeostasis. What are the mitochondrial and nuclear targets of PKCβ physiologically relevant to energy homeostasis? How is the dietary lipid signals transmitted to the PKCβ promoter? Is PKCβ regulatory signaling network dysregulated in metabolic disease states? Can PKCβ inhibition be adopted to prevent human obesity? These important questions should be the target of future studies. The manipulation of PKCβ levels, activity, or signaling might represent a therapeutic approach to combat obesity and associated metabolic disorders.

REFERENCES

1 **Parker PJ**. Protein kinase C: a structurally related family of enzymes Protein kinase C: current concepts and future perspectives. Epand RM, Lester DS, editors. Ellis Horwood, 1992: 3-24
2 **Nishizuka Y**. Protein kinase C and lipid signaling for sustained cellular responses. *FASEB J* 1995; **9**: 484-496 [PMID: 7737456]
3 **Newton AC**. Protein kinase C: structure, function, and regulation. *J Biol Chem* 1995; **270**: 28495-28498 [DOI: 10.1074/jbc.270.48.28495]
4 **Mochly-Rosen D**, Khaner H, Lopez J. Identification of intracellular receptor proteins for activated protein kinase C. *Proc Natl Acad Sci USA* 1991; **88**: 3997-4000 [PMID: 1850844]
5 **Ono Y**, Kikkawa U, Ogita K, Fujii T, Kurokawa T, Asaoka Y, Sekiguchi K, Ase K, Igarashi K, Nishizuka Y. Expression and properties of two types of protein kinase C: alternative splicing from a single gene. *Science* 1987; **236**: 1116-1120 [PMID: 3576226 DOI: 10.1126/science.3576226]
6 **Parker PJ**, Parkinson SJ. AGC protein kinase phosphorylation and protein kinase C. *Biochem Soc Trans* 2001; **29**: 860-863 [PMID: 11709088]
7 **Newton AC**. Regulation of the ABC kinases by phosphory-

lation: protein kinase C as a paradigm. *Biochem J* 2003; **370**: 361-371 [PMID: 12495431]
8 **Newton AC**. Lipid activation of protein kinases. *J Lipid Res* 2009; **50** Suppl: S266-S271 [PMID: 19033211 DOI: 10.1194/jlr.R800064-JLR200]
9 **Sarbassov DD**, Ali SM, Kim DH, Guertin DA, Latek RR, Erdjument-Bromage H, Tempst P, Sabatini DM. Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. *Curr Biol* 2004; **14**: 1296-1302 [PMID: 15268862 DOI: 10.1016/j.cub.2004.06.054]
10 **Ikenoue T**, Inoki K, Yang Q, Zhou X, Guan KL. Essential function of TORC2 in PKC and Akt turn motif phosphorylation, maturation and signalling. *EMBO J* 2008; **27**: 1919-1931 [PMID: 18566587 DOI: 10.1038/emboj.2008.119]
11 **Gould CM**, Kannan N, Taylor SS, Newton AC. The chaperones Hsp90 and Cdc37 mediate the maturation and stabilization of protein kinase C through a conserved PXXP motif in the C-terminal tail. *J Biol Chem* 2009; **284**: 4921-4935 [PMID: 19091746]
12 **Chen D**, Gould C, Garza R, Gao T, Hampton RY, Newton AC. Amplitude control of protein kinase C by RINCK, a novel E3 ubiquitin ligase. *J Biol Chem* 2007; **282**: 33776-33787 [PMID: 17893151 DOI: 10.1074/jbc.M703320200]
13 **Rimessi A**, Rizutto R, Pinton P. Differential recruitment of PKC isoforms in HeLa cells during redox stress. *Cell Stress and Chaperons* 2007; **12**: 291-298 [PMID: 18229448 DOI: 10.1379/csc-211.1]
14 **Scivittaro V**, Ganz MB, Weiss MF. AGEs induce oxidative stress and activate protein kinase C-beta(II) in neonatal mesangial cells. *Am J Physiol Renal Physiol* 2000; **278**: F676-F683 [PMID: 10751230]
15 **Almeida M**, Han L, Ambrogini E, Bartell SM, Manolagas SC. Oxidative stress stimulates apoptosis and activates NF-kappaB in osteoblastic cells via a PKCbeta/p66shc signaling cascade: counter regulation by estrogens or androgens. *Mol Endocrinol* 2010; **24**: 2030-2037 [PMID: 20685851 DOI: 10.1210/me.2010-0189]
16 **Giorgi C**, Agnoletto C, Baldini C, Bononi A, Bonora M, Marchi S, Missiroli S, Patergnani S, Poletti F, Rimessi A, Zavan B, Pinton P. Redox control of protein kinase C: cell- and disease-specific aspects. *Antioxid Redox Signal* 2010; **13**: 1051-1085 [PMID: 20136499 DOI: 10.1089/ars.2009.2825]
17 **Gopalakrishna R**, Jaken S. Protein kinase C signaling and oxidative stress. *Free Radic Biol Med* 2000; **28**: 1349-1361 [PMID: 10924854 DOI: 10.1016/S0891-5849(00)00221-5]
18 **Lin YL**, Shivji MK, Chen C, Kolodner R, Wood RD, Dutta A. The evolutionary conserved zinc finger motif in the largest subunit of human protein kinase A is required for DNA replication and mismatch repair but not for nucleotide excision repair. *J Biol Chem* 1998; **3**: 1453-1461 [PMID: 9430682 DOI: 10.1074/jbc.233.3.1453]
19 **Huang W**, Bansode R, Mehta M, Mehta KD. Loss of protein kinase Cbeta function protects mice against diet-induced obesity and development of hepatic steatosis and insulin resistance. *Hepatology* 2009; **49**: 1525-1536 [PMID: 19296465 DOI: 10.1002/hep.22815]
20 **Obeid LM**, Blobe GC, Karolak LA, Hannun YA. Cloning and characterization of the major promoter of the human protein kinase C beta gene. Regulation by phorbol esters. *J Biol Chem* 1992; **267**: 20804-20810 [PMID: 1400396]
21 **Niino YS**, Ohno S, Suzuki K. Positive and negative regulation of the transcription of the human protein kinase C beta gene. *J Biol Chem* 1992; **267**: 6158-6163 [PMID: 1556124]
22 **Mahajna J**, King P, Parker P, Haley J. Autoregulation of cloned human protein kinase C beta and gamma gene promoters in U937 cells. *DNA Cell Biol* 1995; **14**: 213-222 [PMID: 7880442]
23 **Hagiwara K**, Ito H, Murate T, Miyata Y, Ohashi H, Nagai H. PROX1 overexpression inhibits protein kinase C beta II transcription through promoter DNA methylation. *Genes Chromosomes Cancer* 2012; **51**: 1024-1036 [PMID: 22833470 DOI: 10.1002/gcc.21985]
24 **Bansode RR**, Huang W, Roy SK, Mehta M, Mehta KD. Protein kinase C deficiency increases fatty acid oxidation and reduces fat storage. *J Biol Chem* 2008; **283**: 231-236 [PMID: 17962198]
25 **Yamamoto T**, Watanabe K, Inoue N, Nakagawa Y, Ishigaki N, Matsuzaka T, Takeuchi Y, Kobayashi K, Yatoh S, Takahashi A, Suzuki H, Yahagi N, Gotoda T, Yamada N, Shimano H. Protein kinase Cbeta mediates hepatic induction of sterol-regulatory element binding protein-1c by insulin. *J Lipid Res* 2010; **51**: 1859-1870 [PMID: 20179320 DOI: 10.1194/jlr.M004234]
26 **Huang W**, Bansode RR, Bal NC, Mehta M, Mehta KD. Protein kinase Cβ deficiency attenuates obesity syndrome of ob/ob mice by promoting white adipose tissue remodeling. *J Lipid Res* 2012; **53**: 368-378 [PMID: 22210924 DOI: 10.1194/jlr.M019687]
27 **Kajimura S**, Seale P, Spiegelman BM. Transcriptional control of brown fat development. *Cell Metab* 2010; **11**: 257-262 [PMID: 20374957 DOI: 10.1016/j.cmet.2010.03.005]
28 **Seale P**, Kajimura S, Spiegelman BM. Transcriptional control of brown adipocyte development and physiological function--of mice and men. *Genes Dev* 2009; **23**: 788-797 [PMID: 19339685 DOI: 10.1101/gad.1779209]
29 **Hansen JB**, Kristiansen K. Regulatory circuits controlling white versus brown adipocyte differentiation. *Biochem J* 2006; **398**: 153-168 [PMID: 16898874 DOI: 10.1042/BJ20060402]
30 **Wu J**, Cohen P, Spiegelman BM. Adaptive thermogenesis in adipocytes: is beige the new brown? *Genes Dev* 2013; **27**: 234-250 [PMID: 23388824 DOI: 10.1101/gad.211649.112]
31 **Li Z**, Vaidya VA, Alvaro JD, Iredale PA, Hsu R, Hoffman G, Fitzgerald L, Curran PK, Machida CA, Fishman PH, Duman RS. Protein kinase C-mediated down-regulation of beta1-adrenergic receptor gene expression in rat C6 glioma cells. *Mol Pharmacol* 1998; **54**: 14-21 [PMID: 9658185 DOI: 10.1124/mol.54.1.14]
32 **Fève B**, Elhadri K, Quignard-Boulangé A, Pairault J. Transcriptional down-regulation by insulin of the beta 3-adrenergic receptor expression in 3T3-F442A adipocytes: a mechanism for repressing the cAMP signaling pathway. *Proc Natl Acad Sci USA* 1994; **91**: 5677-5681 [PMID: 8202547]
33 **Spencer MJ**, Croall DE, Tidball JG. Calpains are activated in necrotic fibers from mdx dystrophic mice. *J Biol Chem* 1995; **270**: 10909-10914 [PMID: 7738032]
34 **Pavan C**, Vindigni V, Michelotto L, Rimessi A, Abatangelo G, Cortivo R, Pinton P, Zavan B. Weight gain related to treatment with atypical antipsychotics is due to activation of PKC-β. *Pharmacogenomics J* 2010; **10**: 408-417 [PMID: 20029385 DOI: 10.1038/tps.2009.67]
35 **Zhou Y**, Wang D, Li F, Shi J, Song J. Different roles of protein kinase C-betaI and -delta in the regulation of adipocyte differentiation. *Int J Biochem Cell Biol* 2006; **38**: 2151-2163 [PMID: 16950644 DOI: 10.1016/j.biocel.2006.06.009]
36 **Kleiman E**, Carter G, Ghansah T, Patel NA, Cooper DR. Developmentally spliced PKCbetaII provides a possible link between mTORC2 and Akt kinase to regulate 3T3-L1 adipocyte insulin-stimulated glucose transport. *Biochem Biophys Res Commun* 2009; **388**: 554-559 [PMID: 19686698 DOI: 10.1016/j.bbrc.2009.08.063]
37 **Liberman Z**, Plotkin B, Tennenbaum T, Eldar-Finkelman H. Coordinated phosphorylation of insulin receptor substrate-1 by glycogen synthase kinase-3 and protein kinase C betaII in the diabetic fat tissue. *Am J Physiol Endocrinol Metab* 2008; **294**: E1169-E1177 [PMID: 18430969 DOI: 10.1152/ajpendo.00050.2008]
38 **Osterhoff MA**, Heuer S, Pfeiffer M, Tasic J, Kaiser S, Isken F, Spranger J, Weickert MO, Möhlig M, Pfeiffer AF. Identification of a functional protein kinase Cbeta promoter poly-

morphism in humans related to insulin resistance. *Mol Genet Metab* 2008; **93**: 210-215 [PMID: 17950644 DOI: 10.1016/j.ymgme.2007.09.004]
39 **Sun K**, Kusminski CM, Scherer PE. Adipose tissue remodeling and obesity. *J Clin Invest* 2011; **121**: 2094-2101 [PMID: 21633177 DOI: 10.1172/JCI45887]
40 **Cao Y**. Angiogenesis modulates adipogenesis and obesity. *J Clin Invest* 2007; **117**: 2362-2368 [PMID: 17786229 DOI: 10.1172/JCI32239]
41 **Amadio M**, Bucolo C, Leggio GM, Drago F, Govoni S, Pascale A. The PKCbeta/HuR/VEGF pathway in diabetic retinopathy. *Biochem Pharmacol* 2010; **80**: 1230-1237 [PMID: 20599775 DOI: 10.1016/j.bcp.2010.06.033]
42 **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]
43 **Hennige AM**, Heni M, Machann J, Staiger H, Sartorius T, Hoene M, Lehmann R, Weigert C, Peter A, Bornemann A, Kroeber S, Pujol A, Franckhauser S, Bosch F, Schick F, Lammers R, Häring HU. Enforced expression of protein kinase C in skeletal muscle causes physical inactivity, fatty liver and insulin resistance in the brain. *J Cell Mol Med* 2010; **14**: 903-913 [PMID: 20569275 DOI: 10.1111/j.1582-4934.2008.00629.x]
44 **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/JCI21625]
45 **Curtis JM**, Grimsrud PA, Wright WS, Xu X, Foncea RE, Graham DW, Brestoff JR, Wiczer BM, Ilkayeva O, Cianflone K, Muoio DE, Arriaga EA, Bernlohr DA. Downregulation of adipose glutathione S-transferase A4 leads to increased protein carbonylation, oxidative stress, and mitochondrial dysfunction. *Diabetes* 2010; **59**: 1132-1142 [PMID: 20150287 DOI: 10.2337/db09-1105]
46 **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]
47 **Anderson EJ**, Lustig ME, Boyle KE, Woodlief TL, Kane DA, Lin CT, Price JW, Kang L, Rabinovitch PS, Szeto HH, Houmard JA, Cortright RN, Wasserman DH, Neufer PD. Mitochondrial H2O2 emission and cellular redox state link excess fat intake to insulin resistance in both rodents and humans. *J Clin Invest* 2009; **119**: 573-581 [PMID: 19188683 DOI: 10.1172/JCI37048]
48 **Lee H**, Lee YJ, Choi H, Ko EH, Kim JW. Reactive oxygen species facilitate adipocyte differentiation by accelerating mitotic clonal expansion. *J Biol Chem* 2009; **284**: 10601-10609 [PMID: 19237544 DOI: 10.1074/jbc.M808742200]
49 **Park HS**, Kim SH, Kim YS, Ryu SY, Hwang JT, Yang HJ, Kim GH, Kwon DY, Kim MS. Luteolin inhibits adipogenic differentiation by regulating PPARgamma activation. *Biofactors* 2009; **35**: 373-379 [PMID: 19353690 DOI: 10.1002/biof.38]
50 **Calzadilla P**, Sapochnik D, Cosentino S, Diz V, Dicelio L, Calvo JC, Guerra LN. N-acetyl-cysteine reduces markers of differentiation in 3T3-L1 adipocytes. *Int J Mol Sci* 2011; **12**: 6936-6951 [PMID: 22072928 DOI: 10.3390/ijms12106936]
51 **Kim JR**, Ryu HH, Chung HJ, Lee JH, Kim SW, Kwun WH, Baek SH, Kim JH. Association of anti-obesity activity of N-acetylcysteine with metallothionein-II down-regulation. *Exp Mol Med* 2006; **38**: 162-172 [PMID: 16672770 DOI: 10.1038/emm.2006.20]
52 **Carpéné C**, Iffiú-Soltesz Z, Bour S, Prévot D, Valet P. Reduction of fat deposition by combined inhibition of monoamine oxidases and semicarbazide-sensitive amine oxidases in obese Zucker rats. *Pharmacol Res* 2007; **56**: 522-530 [PMID: 17977742]
53 **Pinton P**, Rimessi A, Marchi S, Orsini F, Migliaccio E, Giorgio M, Contursi C, Minucci S, Mantovani F, Wieckowski MR, Del Sal G, Pelicci PG, Rizzuto R. Protein kinase C beta and prolyl isomerase 1 regulate mitochondrial effects of the lifespan determinant p66Shc. *Science* 2007; **315**: 659-663 [PMID: 17272725 DOI: 10.1126/science.1135380]
54 **Pinton P**, Rizzuto R. p66Shc, oxidative stress and aging: importing a lifespan determinant into mitochondria. *Cell Cycle* 2008; **7**: 304-308 [PMID: 18235239]
55 **Patergnani S**, Marchi S, Rimessi A, Bonora M, Giorgi C, Mehta KD, Pinton P. PRKCB/protein kinase C, beta and the mitochondrial axis as key regulators of autophagy. *Autophagy* 2013; **9**: 1367-1385 [PMID: 23778835 DOI: 10.4161/auto.25239]
56 **Kim KH**, Jeong YT, Oh H, Kim SH, Cho JM, Kim YN, Kim SS, Kim do H, Hur KY, Kim HK, Ko T, Han J, Kim HL, Kim J, Back SH, Komatsu M, Chen H, Chan DC, Konishi M, Itoh N, Choi CS, Lee MS. Autophagy deficiency leads to protection from obesity and insulin resistance by inducing Fgf21 as a mitokine. *Nat Med* 2013; **19**: 83-92 [PMID: 23202295]
57 **Yang L**, Li P, Fu S, Calay ES, Hotamisligil GS. Defective hepatic autophagy in obesity promotes ER stress and causes insulin resistance. *Cell Metab* 2010; **11**: 467-478 [PMID: 20519119 DOI: 10.1016/j.cmet.2010.04.005]
58 **Singh R**, Xiang Y, Wang Y, Baikati K, Cuervo AM, Luu YK, Tang Y, Pessin JE, Schwartz GJ, Czaja MJ. Autophagy regulates adipose mass and differentiation in mice. *J Clin Invest* 2009; **119**: 3329-3339 [PMID: 19855132 DOI: 10.1172/JCI39228]
59 **Zhang Y**, Goldman S, Baerga R, Zhao Y, Komatsu M, Jin S. Adipose-specific deletion of autophagy-related gene 7 (atg7) in mice reveals a role in adipogenesis. *Proc Natl Acad Sci USA* 2009; **106**: 19860-19865 [PMID: 19910529 DOI: 10.1073/pnas.0906048106]
60 **Nuñez CE**, Rodrigues VS, Gomes FS, Moura RF, Victorio SC, Bombassaro B, Chaim EA, Pareja JC, Geloneze B, Velloso LA, Araujo EP. Defective regulation of adipose tissue autophagy in obesity. *Int J Obes* (Lond) 2013; **37**: 1473-1480 [PMID: 23478428 DOI: 10.1038/ijo.2013.27]
61 **Jansen HJ**, van Essen P, Koenen T, Joosten LA, Netea MG, Tack CJ, Stienstra R. Autophagy activity is up-regulated in adipose tissue of obese individuals and modulates proinflammatory cytokine expression. *Endocrinology* 2012; **153**: 5866-5874 [PMID: 23117929 DOI: 10.1210/en.2012-1625]
62 **He C**, Bassik MC, Moresi V, Sun K, Wei Y, Zou Z, An Z, Loh J, Fisher J, Sun Q, Korsmeyer S, Packer M, May HI, Hill JA, Virgin HW, Gilpin C, Xiao G, Bassel-Duby R, Scherer PE, Levine B. Exercise-induced BCL2-regulated autophagy is required for muscle glucose homeostasis. *Nature* 2012; **481**: 511-515 [PMID: 22258505 DOI: 10.1038/nature10758]
63 **Dekker LV**, Leitges M, Altschuler G, Mistry N, McDermott A, Roes J, Segal AW. Protein kinase C-beta contributes to NADPH oxidase activation in neutrophils. *Biochem J* 2000; **1**: 285-289 [PMID: 10727429]
64 **Gray RD**, Lucas CD, Mackellar A, Li F, Hiersemenzel K, Haslett C, Davidson DJ, Rossi AG. Activation of conventional protein kinase C (PKC) is critical in the generation of human neutrophil extracellular traps. *J Inflamm* (Lond) 2013; **10**: 12 [PMID: 23514610 DOI: 10.1186/1476-9255-10-12]
65 **Kitada M**, Koya D, Sugimoto T, Isono M, Araki S, Kashiwagi A, Haneda M. Translocation of glomerular p47phox and p67phox by protein kinase C-beta activation is required for oxidative stress in diabetic nephropathy. *Diabetes* 2003; **52**: 2603-2614 [PMID: 14514646 DOI: 10.2337/diabetes.52.10.2603]
66 **Scherz-Shouval R**, Elazar Z. Regulation of autophagy by ROS: physiology and pathology. *Trends Biochem Sci* 2011; **36**: 30-38 [PMID: 20728362 DOI: 10.1016/j.tibs.2010.07.007]
67 **Huang J**, Canadien V, Lam GY, Steinberg BE, Dianauer MC,

Magalhaes MAO, Giogauer M, Grinstein S, Brumell JH. Activation of antibacterial autophagy by NADPH oxidases. *Proc Natl Acad Sci USA* 2009; **106**: 6226-6231 [PMID: 19339495 DOI: 10.1073/pnas.0811045106]

68 **Goss VL**, Hocevar BA, Thompson LJ, Stratton CA, Burns DJ, Fields AP. Identification of nuclear beta II protein kinase C as a mitotic lamin kinase. *J Biol Chem* 1994; **269**: 19074-19080 [PMID: 8034666]

69 **Metzger E**, Imhof A, Patel D, Kahl P, Hoffmeyer K, Friedrichs N, Müller JM, Greschik H, Kirfel J, Ji S, Kunowska N, Beisenherz-Huss C, Günther T, Buettner R, Schüle R. Phosphorylation of histone H3T6 by PKCbeta(I) controls demethylation at histone H3K4. *Nature* 2010; **464**: 792-796 [PMID: 20228790 DOI: 10.1038/nature08839]

70 **Teng H**, Ballim RD, Mowla S, Prince S. Phosphorylation of histone H3 by protein kinase C signaling plays a critical role in the regulation of the developmentally important TBX2 gene. *J Biol Chem* 2009; **284**: 26368-26376 [PMID: 19633291 DOI: 10.1074/jbc.M109.021360]

71 **Clarke DL**, Sutcliffe A, Deacon K, Bradbury D, Corbett L, Knox AJ. PKCbetaII augments NF-kappaB-dependent transcription at the CCL11 promoter via p300/CBP-associated factor recruitment and histone H4 acetylation. *J Immunol* 2008; **181**: 3503-3514 [PMID: 18714023]

72 **Hsieh JC**, Jurutka PW, Nakajima S, Galligan MA, Haussler CA, Shimizu Y, Shimizu N, Whitfield GK, Haussler MR. Phosphorylation of the human vitamin D receptor by protein kinase C. Biochemical and functional evaluation of the serine 51 recognition site. *J Biol Chem* 1993; **268**: 15118-15126 [PMID: 8392065]

73 **Suzuma K**, Takahara N, Suzuma I, Isshiki K, Ueki K, Leitges M, Aiello LP, King GL. Characterization of protein kinase C beta isoform's action on retinoblastoma protein phosphorylation, vascular endothelial growth factor-induced endothelial cell proliferation, and retinal neovascularization. *Proc Natl Acad Sci USA* 2002; **99**: 721-726 [PMID: 11805327 DOI: 10.1073/pnas.022644499]

74 **Sauma S**, Yan Z, Ohno S, Friedman E. Protein kinase C beta 1 and protein kinase C beta 2 activate p57 mitogen-activated protein kinase and block differentiation in colon carcinoma cells. *Cell Growth Differ* 1996; **7**: 587-594 [PMID: 8732668]

75 **Formisano P**, Oriente F, Fiory F, Caruso M, Miele C, Maitan MA, Andreozzi F, Vigliotta G, Condorelli G, Beguinot F. Insulin-activated protein kinase Cbeta bypasses Ras and stimulates mitogen-activated protein kinase activity and cell proliferation in muscle cells. *Mol Cell Biol* 2000; **20**: 6323-6333 [PMID: 10938109]

76 **Patel NA**, Yamamoto M, Illingworth P, Mancu D, Mebert K, Chappell DS, Watson JE, Cooper DR. Phosphoinositide 3-kinase mediates protein kinase C beta II mRNA destabilization in rat A10 smooth muscle cell cultures exposed to high glucose. *Arch Biochem Biophys* 2002; **403**: 111-120 [PMID: 12061808]

77 **Naruse K**, Rask-Madsen C, Takahara N, Ha SW, Suzuma K, Way KJ, Jacobs JR, Clermont AC, Ueki K, Ohshiro Y, Zhang J, Goldfine AB, King GL. Activation of vascular protein kinase C-beta inhibits Akt-dependent endothelial nitric oxide synthase function in obesity-associated insulin resistance. *Diabetes* 2006; **55**: 691-698 [PMID: 16505232 DOI: 10.2337/diabetes.55.03.06.db05-0771]

78 **Wu D**, Peng F, Zhang B, Ingram AJ, Kelly DJ, Gilbert RE, Gao B, Krepinsky JC. PKC-beta1 mediates glucose-induced Akt activation and TGF-beta1 upregulation in mesangial cells. *J Am Soc Nephrol* 2009; **20**: 554-566 [PMID: 19211711 DOI: 10.1681/ASN.2008040445]

79 **Guo K**, Liu Y, Zhou H, Dai Z, Zhang J, Sun R, Chen J, Sun Q, Lu W, Kang X, Chen P. Involvement of protein kinase C beta-extracellular signal-regulating kinase 1/2/p38 mitogen-activated protein kinase-heat shock protein 27 activation in hepatocellular carcinoma cell motility and invasion. *Cancer Sci* 2008; **99**: 486-496 [PMID: 18167130 DOI: 10.1111/j.1349-7006.2007.00702.x]

80 **Patti ME**, Corvera S. The role of mitochondria in the pathogenesis of type 2 diabetes. *Endocr Rev* 2010; **31**: 364-395 [PMID: 20156986 DOI: 10.1210/er.2009-0027]

81 **Kusminski CM**, Scherer PE. Mitochondrial dysfunction in white adipose tissue. *Trends Endocrinol Metab* 2012; **23**: 435-443 [PMID: 22784416 DOI: 10.1016/j.tem.2012.06.004]

82 **Choo HJ**, Kim JH, Kwon OB, Lee CS, Mun JY, Han SS, Yoon YS, Yoon G, Choi KM, Ko YG. Mitochondria are impaired in the adipocytes of type 2 diabetic mice. *Diabetologia* 2006; **49**: 784-791 [PMID: 16501941 DOI: 10.1007/s00125-006-0170-2]

83 **Rong JX**, Qiu Y, Hansen MK, Zhu L, Zhang V, Xie M, Okamoto Y, Mattie MD, Higashiyama H, Asano S, Strum JC, Ryan TE. Adipose mitochondrial biogenesis is suppressed in db/db and high-fat diet-fed mice and improved by rosiglitazone. *Diabetes* 2007; **56**: 1751-1760 [PMID: 17456854 DOI: 10.2337/db06-1135]

84 **Dahlman I**, Forsgren M, Sjögren A, Nordström EA, Kaaman M, Näslund E, Attersand A, Arner P. Downregulation of electron transport chain genes in visceral adipose tissue in type 2 diabetes independent of obesity and possibly involving tumor necrosis factor-alpha. *Diabetes* 2006; **55**: 1792-1799 [PMID: 16731844 DOI: 10.2337/db05-1421]

85 **Bogacka I**, Ukropcova B, McNeil M, Gimble JM, Smith SR. Structural and functional consequences of mitochondrial biogenesis in human adipocytes in vitro. *J Clin Endocrinol Metab* 2005; **90**: 6650-6656 [PMID: 16204368 DOI: 10.1210/jc.2005-1024]

86 **Wilson-Fritch L**, Nicoloro S, Chouinard M, Lazar MA, Chui PC, Leszyk J, Straubhaar J, Czech MP, Corvera S. Mitochondrial remodeling in adipose tissue associated with obesity and treatment with rosiglitazone. *J Clin Invest* 2004; **114**: 1281-1289 [PMID: 15520860 DOI: 10.1172/JCI21752]

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Diabetic nephropathy and inflammation

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Abstract

Diabetic nephropathy (DN) is the leading cause of end-stage renal failure worldwide. Besides, diabetic nephropathy is associated with cardiovascular disease, and increases mortality of diabetic patients. Several factors are involved in the pathophysiology of DN, including metabolic and hemodynamic alterations, oxidative stress, and activation of the renin-angiotensin system. In recent years, new pathways involved in the development and progression of diabetic kidney disease have been elucidated; accumulated data have emphasized the critical role of inflammation in the pathogenesis of diabetic nephropathy. Expression of cell adhesion molecules, growth factors, chemokines and pro-inflammatory cytokines are increased in the renal tissues of diabetic patients, and serum and urinary levels of cytokines and cell adhesion molecules, correlated with albuminuria. In this paper we review the role of inflammation in the development of diabetic nephropathy, discussing some of the major inflammatory cytokines involved in the pathogenesis of diabetic nephropathy, including the role of adipokines, and take part in other mediators of inflammation, as adhesion molecules.

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Key words: Diabetic Nephropathy; Inflammation; Albuminuria; Adhesion molecules; Cytokines

Core tip: In recent years, new pathways involved in the development and progression of diabetic kidney disease have been elucidated; accumulated data have emphasized the critical role of inflammation in its pathogenesis. Expression of cell adhesion molecules, growth factors, chemokines and pro-inflammatory cytokines increased in renal tissues of diabetic patients, and serum and urinary levels of cytokines and cell adhesion molecules, correlated with albuminuria. We review the role of inflammation in the development of diabetic nephropathy, discussing some of the major inflammatory cytokines involved in its pathogenesis, including the role of adipokines, and other mediators of inflammation, as adhesion molecules.

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INTRODUCTION

Diabetes mellitus (DM) is the leading cause of chronic renal failure in development countries and is increasing as a cause of morbility and mortality worldwide. Both type 1 and 2 diabetes, but principally the last one, plays an important role in this problem because of the impact of its complications[1-4].

Among all these complications, diabetic nephropathy (DN) has become the principal cause of end-stage renal failure and cardiovascular mortality, this condition appears after many years of diabetes beginning[3,5].

It is well understood that type-2 DM is not an immune disease but at this time we could consider that there is evidence that the combine of immunologic and inflammatory mechanisms play a pivotal role in its presentation, development and finally its progression.

The DN take place nearby one-third of patient with type 1 DM and 25% approximately of patients with type 2[4,6].

In México, it is described that the main cause of chronic renal failure is type 2 DM, nevertheless we know that not all diabetic patients develop DN, moreover glucose control is not a warranty of a life free of microangiopathic complications[7].

It has been found that despite all pharmacologic therapies available for DN treatment, some patients develop kidney damage, that is why the need of complete understanding of molecular, metabolic and environmental factors that lead to DN and their interaction between them.

Among diverse factors that could interact actively in pathogenesis and progression of DN have been studied the age, gender, smoking, hypertension and hyperuricemia, all of them with suggestive results of correlation with renal disease[2].

In this paper we review the inflammatory factors that lead to the development and progression of DN.

PHYSIOPATHOLOGY

DN is characterized by glomerular hypertrophy, thickness of basement, tubular and glomerular membranes and accumulation of extracellular matrix in these membranes that finally cause tubulointerstitial and glomerular fibrosis and sclerosis[2,6,8]. As we can see several kidney structures are susceptible to hyperglycemia, and this metabolic change cause organ damage due to several cellular *via* including genetic activation and expression, advanced glycation end products generation, polyol pathway activation, abnormal protein kinase activation (PKC), raise of oxidative stress and the molecules that act as growth factors, transcription factors and others[4,8].

There is a response for hyperglycemia from the system, the transcription factors regulate the gene encoding some cytokines like transforming growth factor β (TGF-β), chemokine C-C motif ligand 2, fibronectin, osteopontin, decorin, thrombospondin, aldose reductase and plasminogen activator inhibitor 1, all these molecules involved in inflammation, extracellular matrix synthesis and its degradation are increased in type-2 DM[4].

Some other factors in relation to DN, it is known that some metabolic *via* activated by hyperglycemia are not enough to cause the kidney complication. The family predisposition to disease, race and other environmental factors interact with hemodynamic changes producing, as a result, advanced glycation end products, glucose reduction and sorbitol accumulation into the cell, overproduction of reactive oxygen species and activation of signaling *via* as PKC and mitogen-activated protein kinase[2].

Diabetic patients then could have albuminuria since early phases or stages of organ damage, it is also considered as a very sensible marker of kidney disease progression. As a result there are many glomerular abnormalities including podocyte structure alteration, reduction of nephrin expression and increase of filtration rate, a hallmark of DN[9].

Many mechanisms were investigated in this process, for a better understanding these are divided in mechanisms of immune cell infiltration of kidney, molecules involved in progression and intracellular pathways activated in DN.

Role of inflammation

Now we know that activation of the immune system and chronic inflammation are both involved in pathogenesis of DM and as a result DN. Some studies have demonstrated that cytokines, chemokines, growth factors, adhesion molecules, nuclear factors as well as immune cells as monocytes, lymphocytes and macrophages are all involved in DM pathogenesis and of course play an important role in DM complications[1,5].

IMMUNE CELLS

Macrophages

Macrophages are recognized as the principal inflammatory cell involved in kidney damage, their accumulation relates with severity of DN in experimental models[3].

These cells are responsible of the calling "renal remodeling", so therapeutics proposed to inhibit their accumulation may help to stop progression.

Two subtypes are mainly involved in DN, M1 macrophages activated by Th1 cells, that are able to increase inflammatory response by cytokines expression [interleukins, tumor necrosis factor (TNF) and interferon γ]; and M2 macrophages activated by Th2 cells that promote tissue repairmen, remodeling and neovascularization by antiinflammatory cytokines expression[3]. Is in this way that investigations are working, it is known that the macrophage subtype levels related with recruitment of circulating monocytes from vascular space to glomerular tissue.

Meanwhile M1 macrophages enhance inflammatory response by upper production of reactive oxygen species (ROS), this point will be reviewed later.

As to activated M2 macrophages, they help in inflammation ending with the participation of interleukin 10 (IL-10), TGF-β1, both with anti-inflammatory functions. Besides they produce proinflammatory factors as chemokines, cytokines and superoxide anions[3].

Many investigations are directed to show that statins are capable to block M1 macrophage actions but at the same time improve M2 functions. It will be helpful as one of the strategies used in the treatment of DN directed to this point.

T lymphocytes

T lymphocytes play a determinant role in early kidney damage in DN, they have cytotoxic effects besides macrophages tissue activation[3].

The first contribution of the studies was about the increase in local accumulating T cells in diabetic experimental models. Xiao *et al*[10] and Moon *et al*[11] showed an increase in CD4 and CD8 lymphocytes in diabetic mouse, these changes were observed in glomeruli and interstice.

In type 1 DM there is an increase of T lymphocytes

in juxtaglomerular tissue that results in a disturbance in albumin glomerular excretion and a decrease of renal filtration. Many other studies have shown at this time that T lymphocytes systemic, specifically circulating CD8, correlated with albuminuria[6].

Lei *et al*[6] demonstrated with a multiple regression analysis a positive association between lymphocytes CD8 and albuminuria in type 2 DM patients and the cell activation could be a systemic response.

Several metabolic and genetic *via*, may activate systemic T lymphocytes. In type 2 DM those cells may be activated by hemodynamic, environmental and metabolic changes. The most important activation seen due to hyperglycemia, that activates nuclear factor κB and this results in an over stimulation of lymphocytes by specific cytokines as IL-12 produced by macrophages, and then, production of interferon further lymphocyte activation[6].

CHEMOKINES

These molecules are active components of inflammatory cells recruitment in kidney and are present in every phase of kidney damage[8].

Many chemokines are involved in the inflammatory response in DN, monocyte chemoattractant protein (MCP-1) was first described in its role in early phases of atherosclerosis[12].

MCP-1

MCP-1 can promote transformation of monocytes in macrophages, the last ones produce diverse cytokines as IL-6 and TNF-α, both induce atherosclerosis changes in vascular walls that results in illness progression. Because of its expression is as high in the atherosclerotic plaques than in impaired plaques, systemic MCP-1 was measured in many studies in order to show an association between this chemokine and DN markers. Takebayashi *et al*[12] found that patients with urinary albumin excretion presented higher circulating levels of MCP-1 than patients without this alteration.

All these findings could suggest that MCP-1 plays an important role in pathogenesis of DN as the protein produced not only in vascular wall, atherosclerotic plaques but also in tubular epithelial cells.

CYTOKINES

Cytokines are molecules with a wide spectrum of physiological actions, many of them due to their pleiotropic actions. They have capacity to combine actions in order to amplify their effects and then induce synthesis or expression of other cytokines if needed.

In 1991 it was suggested for the first time the participation of cytokines with inflammatory actions in the development of DN, by demonstration of high production of these molecules from macrophages in glomerular membranes from diabetic rats, but not from non-diabetic rats[5].

At this time we now that inflammatory cytokines play an important role in DN, but cytokines have been involved in the development of other microangiopathic complications of DM[1].

Interleukins

Interleukins are a group of cytokines produced by many cells in different tissues. According to their physiologic actions, they are classified as antiinflammatory and proinflammatory molecules[3].

IL-1

Many studies have shown that IL-1 promotes an increase of adhesion molecules in glomerular endothelium as well as expression of these molecules in other kidney structures[1].

Mesangial cells and renal tubular epithelium overexpress intercellular adhesion molecule-1 (ICAM-1) and E-selectin, additionally, IL-1 induces prostaglandin E2 synthesis in mesangial cells, this fact cause alterations in the glomerular hemodynamics[1].

Moreover, IL-1 stimulates hyaluronan synthesis, leading to cell proliferation in DM patients, this facts contributes to development of DN. It is known that this proinflammatory cytokin is increased in experimental models with albuminuria and at the same time with macrophages accumulation[1]. According to these pathological changes, IL-1 modifies vascular permeability and increase expression of chemokines that as a result leads proliferation and synthesis of extracellular matrix in mesangium[3].

IL-6

IL-6 is another molecule that has been studied in DN due to its pleiotropic effects. Many authors showed that IL-6 concentration is increased in DN. IL-6 has a direct effect in glomerular and infiltrating cells, this effect modified extracellular matrix dynamics affecting membrane thickening in renal glomeruli[1,3].

IL-6 is a cytokine that can enhance proliferation, overexpression of extracellular matrix and affect vascular permeability; these actions lead to DN progress[1].

It has been shown that serum IL-6 is increased in patients with type 2 DM with nephropathy[3].

IL-18

The principal actions of this inflammatory cytokine are; to enhance the production of other inflammatory cytokines by mesangial cells, and upregulation of ICAM-1. Its serum concentration is increased in DN as well as other interleukins and has a determinant role in endothelium apoptosis[1].

IL-18 has several sources in the diabetic kidney as infiltrating, T-lymphocytes, macrophages, monocytes as well as proximal tubule cells. There is a direct correlation between IL-18, albuminuria and albumin excretion rate, so it's relationship with nephropathy has been identified[13].

TNF-α

This is an inflammatory cytokine with many determinant

actions in inflammatory response by several tissues and pleiotropic effects. TNF-α is produced by infiltrating cells, as monocytes, macrophages and T lymphocytes, as well as kidney cells. Previous reports shown that TNF-α can be stored as a proactive form[1].

Its actions are widely known as systemic and in many cases direct cytotoxic effect in kidney cells principally. Nevertheless actions as activation of second messengers, transcription factors (TF), growth factors, cell adhesion molecules, express or synthesis of cytokines and others are recognized as variable biological effects of this molecule, of course all of them playing a determinant role in DN pathogenesis[1].

When TNF-α binds to the receptors, several signaling pathways are activated and a cascade of molecules begin their expression in renal cells, many of this actions results in apoptosis and necrosis[5].

The negative effects have been described in experimental models and in humans[1]. Those effects were manifested as DM nephropathy, hypertension, nephritis and glomerulonephritis, this fact could be demonstrated with the correlation found by Navarro-González *et al*[5]. in 2005 between renal TNF-α and albumin excretion in diabetic mice. This observation demonstrated that this inflammatory molecule is directly involved in pathogenesis of DN by leading cell and tissue damage; moreover albuminuria has been related to a enhanced stimuli for overexpression of TNF-α[3].

TNF-α alters glomerular hemodynamics and promotes increased vascular endothelium permeability. Infiltration by inflammatory cells, neo-formation of extracellular matrix, production of ROS and blood flow disturb are others recognized effects of TNF-α in renal structures[1].

TGF-β1

TGF-β1 is a cytokine member of TGF-β1 superfamily considered also as a transcription factor related to development of renal damage by promoting renal fibrosis. Its activity is recognized as inflammatory and fibrogenic, with two isoforms, TGF-β2 and TGF-β3, all produced by kidney cells, the union between this cytokine and its receptor phosphorylate the Smads. Smads are intracellular proteins that transduce extracellular signals from TGF-β ligands to the cellular nucleus and activate downstream gene transcription. This family is considered to be involved in development of inflammation and fibrosis in the kidney[4,8,13].

That is why TGF-β1 is recognized as one of the principal mediators of structural changes seen in DN, its concentration is higher in DM patients with urinary albumin excretion than in normal individuals[8].

The upregulation of TGF-β1 promotes extracellular matrix proliferation and at the same time inhibits the degradation, so that is why actually overexpression of this factor is directly associated with severe forms of glomeruloesclerosis and glomerulonephritis[8]. Some other changes are favored by TGF-β1, for example the induction of transforming epithelial cells of tubules into fibroblasts; this process is responsible of renal fibrosis, a result of persistent inflammation.

TGF-β1 is considered too as a cytokine which principal function in inflammation is to inhibit this process. Letterio *et al*[14] discovered that experimental models with impairment in *TGF-β1* gene are highly susceptible to several inflammation resulting in autoimmune diseases and even death[15,16].

El Mesallamy *et al*[8] correlated TGF-β1 concentrations with Connective tissue growth factor level; their findings showed that between these two molecules there is a closed interaction in DN. So as we can see, TGF-β1 is a molecule that can regulate not only its own release and its actions but also it has the ability to modulate other molecular releases and their interactions in signaling pathways.

It seems like TGF-β1 has a complex role in renal inflammation, we know that this protein is present as active and as a latent forms, the first one is related to mediator of renal fibrosis that can progress according to many other factors. The second form is a protective factor for the development of renal damage. Some mechanisms for these findings are not well understood yet[17].

TF

Proteins known as TF bind themselves to some gene specific regions to activate or inhibit nuclear transcription process[4].

TF were classified according to its main action, they can be constitutively active or regulatory factors and they can be activated by several metabolic and environmental stimuli in many cellular sites. Due to this last point we can subclassify TF in nuclear factors, cytoplasmic factors and steroid receptor superfamily[4].

Several TF are involved in DN development, here we have the most relevant.

Upstream stimulatory factors 1 (USF1) and USF2 are a part of Myc family and encoded by two different genes.

USF1 and USF2 are involved in some glucose genes responses in many types of cells including kidney cells. It has been shown that overexpression or increase in concentration of these TF are related with albuminuria development and even more the upregulation of many other molecules with proved actions in DN pathogenesis[4].

Smads

Smads conform a transcription factor family that regulates the expression of certain genes. Three classes are known: the receptor-regulated Smads (R-SMAD) which include SMAD1, SMAD2, SMAD3, SMAD5 and SMAD8/9; the common-mediator Smad (co-SMAD) which includes only SMAD4, which interacts with R-SMADs to take part in signaling and the antagonistic or inhibitory Smads which include SMAD6 and SMAD7, they block the activation of R-SMADs and co-SMADs[17].

As mentioned before this family is closely involved with TGF-β1, which phosphorylate Smad 2 and Smad 3 to form a complex with Smad 4, all this process leads to regulate gene in cell nuclei[17].

Smad 4 is the most related with inflammation, if there is an abnormality of this protein, the inflammatory response is more intense and leads a higher concentration of diverse cytokines and adhesion molecules.

There is another relationship that leads the process to be functional for kidney, this happens when TGF-β1 regulates Smad 7 transcription by Smad 3 and Smad 4 binding, so, when Smad 4 is impaired we can see and exaggerated inflammatory response for reduction of Smad 7 expression, activation of Nuclear Factor κB and fibrosis inhibition[17].

Smad 4 seems to be a key point in regulation of TGF-β1 and its different functions media the conjunction with Smad 7 and Smad 3 expression in kidney.

The case of Smad 7 is quiet interesting, it acts in an inhibitory way and regulates the active function of Smad 2 and Smad 3 but by a negative feedback.

The Smad 7 expression is enhanced by TGF-β1 that in normal condition has a negative feedback inhibit the action of Smad and at the same time degrade this transcription factors. When Smad 7 gets degraded then kidney fibrosis begins. If Smad 7 decline renal inflammation persists and as a result begins fibrosis *via* TGF-β and Smad 3.

In as much as the pivotal role of Smad 7 some investigators decided to study therapeutic effects of this factor in experimental models. When Smad 7 was transferred to kidney they found that if there is an overexpression of Smad 7, inflammation and fibrosis decrease.

Adhesion molecules

ICAM-1 and vascular adhesion molecule-1 (VCAM-1) are involved in the attachment of leukocytes to the vascular wall and penetration into the intima, once there, leukocytes can produce proteolytic enzymes that lead to tissue and organ damage, or differentiate into foam cells that lead to the atherosclerotic process[15].

Several animal models have shown that mice deficient in ICAM-1 are resistant to nephropathy in experimental models of diabetes, while treatment with anti-ICAM-1 monoclonal Ab prevents mononuclear cell infiltration into diabetic glomeruli[3].

Our group has shown that the levels of VCAM-1 correlate with the severity of albuminuria in diabetic hypertensive patients[15]. In addition, Seron *et al*[16] reported that VCAM-1 expression is increased in kidney biopsies from patients with DN, they also found a correlation between levels of VCAM-1 and numbers of infiltrating immune cells[18].

ADIPOKINES

Adiponectin and resistin were first described as adipocyte-secreted hormones (adipocytokines) that modulate insulin action. Both; hypoadiponectinemia and hyperresistinemia are associated with inflammation[19].

Hypoadiponectinemia has been reported as a risk factor for the development of albuminuria in mice[19], whereas in humans, resistin is mainly a monocyte-macrophage product. In humans hyperresistinemia promotes the expression of adhesion molecules[20], and is involved in the pathways that lead to albuminuria and renal damage[21].

WHICH INFLAMMATORY MOLECULE?

Certainly, inflammation is an important player in the pathogenesis of DN, However, because of multiple pathways that joint inflammation with diabetic complications, it looks unlikely that one single molecule be sufficient for the development of DN. It is also true that the blockade of the principal mediators could be useful in the prevention of this complication; several studies have been designed in order to indentify therapeutic targets.

The evidence suggest that TNF-α, MCP-1 and adhesion molecules have a prominent role in the development of DN, and all these mediators may be considered therapeutic targets for the prevention and treatment of DN, as we will discuss in the next section.

PERSPECTIVES

Microinflammation is the most important mechanism for development and progression of DN. Our knowledge related to signaling pathways involved in its pathogenesis has not been elucidated at all.

There are several pivotal mediators of inflammation, and their interactions are determinant in the process.

We have reviewed not only biological actions of these mediators, but also their possible therapeutic effects in experimental models.

The Smad family plays a very important role in inflammation and fibrosis in renal disease, its different actions among all molecular mediators leads to open several optional researches in DN.

A very interesting advanced is that if levels of Smad 7 could be restored in sick kidneys we could balance inflammatory responses in patients with renal diseases.

But not only Smad family could be a therapeutic option for DN patients, at this time it is very important take into a count that gene polymorphisms encoding several molecules in this patients have to be modified. Is in this way that investigations are aimed, looking to stop the progression of the disease, and not just for uncontrolled DM but also for other diseases involving the kidney.

Many options for interfering in transcription factors activation have been proposed, first blocking TF binding and second blocking TF pathways for activation. For these conditions there were used by both TF and experimental molecules.

Several studies are needed for interfering with signaling pathways not just for treatment of an abnormal condition as DN but also to prevent it.

Experimental studies have shown that inhibition of TNF-α (with the use of soluble TNF-α receptor fusion proteins, monoclonal antibodies or pentoxifylline) might be an efficacious treatment for renal disease secondary to diabetes mellitus, being pentoxifylline equivalent in efficacy and safety to captopril, and the addition of than drug to inhibitors of the renin-angiotensin system increases

their antiproteinuric effect[1,5].

Our group found that the reduction of urinary albumin excretion with the use of the fixed dose combination trandolapril-verapamil, depends not only from its antihypertensive effect, but also from its action on VCAM-1 adhesion molecules levels[22].

CONCLUSION

Inflammation plays an essential role in the development of DN, this participation involves increased chemokine production, infiltration of inflammatory cells to the kidney, pro-inflammatory cytokine production and tissue damage.

Several components of the diabetic milieu, as hyperglycemia, renin-angiotensin system and oxidative stress can activate the inflammatory process in the kidneys, which results in the infiltration of the organ by monocytes and lymphocytes, which secrete injurious molecules, such as proinflammatory cytokines and reactive oxygen species.

This leukocyte activity amplifies the inflammatory response and promotes cell injury and the development of fibrosis. Better understanding of the inflammatory response in diabetic kidneys is expected to identify novel anti-inflammatory strategies for the potential treatment of human DN.

REFERENCES

1 **Navarro-González JF**, Mora-Fernández C. The role of inflammatory cytokines in diabetic nephropathy. *J Am Soc Nephrol* 2008; **19**: 433-442 [PMID: 18256353 DOI: 10.1681/ASN.2007091048]

2 **Gaballa M**, Farag MK. Predictors of Diabetic Nephropathy. *Eur J Med* 2013; **8**: 287-296 [DOI: 10.2478/s11536-012-0055-3]

3 **Lopez-Parra V**, Mallavia B, Egido J, Gomez-Guerrero C. Immunoinflammation in Diabetic Nephropathy: Molecular Mechanisms and Therapeutic Options. In: Chan JSD. Diabetic Nephropathy. InTech, Chapters, 2012: 127-146 [DOI: 10.5772/1889]

4 **Sanchez AP**, Sharma K. Transcription factors in the pathogenesis of diabetic nephropathy. *Expert Rev Mol Med* 2009; **11**: e13 [PMID: 19397838]

5 **Navarro-González JF**, Jarque A, Muros M, Mora C, García J. Tumor necrosis factor-alpha as a therapeutic target for diabetic nephropathy. *Cytokine Growth Factor Rev* 2009; **20**: 165-173 [PMID: 19251467 DOI: 10.1016/j.cytogfr.2009.02.005]

6 **Lei L**, Mao Y, Meng D, Zhang X, Cui L, Huo Y, Wang Y. Percentage of circulating CD8+ T lymphocytes is associated with albuminuria in type 2 diabetes mellitus. *Exp Clin Endocrinol Diabetes* 2014; **122**: 27-30 [PMID: 24203650 DOI: 10.1055/s-0033-1358666]

7 **Cueto-Manzano AM**, Cortes-Sanabria L, Martinez-Ramirez HR, Rojas-Campos E, Barragan G, Alfaro G, Flores J, Anaya M, Canales-Munoz JL. Detection of early nephropathy in Mexican patients with type 2 diabetes mellitus. *Kidney Int Suppl* 2005; (**97**): S40-S45 [PMID: 16014099 DOI: 10.1111/j.1523-1755.2005.09707.x]

8 **El Mesallamy HO**, Ahmed HH, Bassyouni AA, Ahmed AS. Clinical significance of inflammatory and fibrogenic cytokines in diabetic nephropathy. *Clin Biochem* 2012; **45**: 646-650 [PMID: 22421318 DOI: 10.1016/j.clinbiochem.2012.02.021]

9 **Cao Z**, Cooper ME. Pathogenesis of diabetic nephropathy. *J Diabetes Invest* 2011; **2**: 243-247 [DOI: 10.1111/j.2040-1124.2011.00131.x]

10 **Xiao X**, Ma B, Dong B, Zhao P, Tai N, Chen L, Wong FS, Wen L. Cellular and humoral immune responses in the early stages of diabetic nephropathy in NOD mice. *J Autoimmun* 2009; **32**: 85-93 [PMID: 19200691 DOI: 10.1016/j.jaut.2008.12.003]

11 **Moon JY**, Jeong KH, Lee TW, Ihm CG, Lim SJ, Lee SH. Aberrant recruitment and activation of T cells in diabetic nephropathy. *Am J Nephrol* 2012; **35**: 164-174 [PMID: 22286547 DOI: 10.1159/000334928]

12 **Takebayashi K**, Matsumoto S, Aso Y, Inukai T. Association between circulating monocyte chemoattractant protein-1 and urinary albumin excretion in nonobese Type 2 diabetic patients. *J Diabetes Complications* 2006; **20**: 98-104 [PMID: 16504838 DOI: 10.1016/j.jdiacomp.2005.05.008]

13 **Nakamura A**, Shikata K, Hiramatsu M, Nakatou T, Kitamura T, Wada J, Itoshima T, Makino H. Serum interleukin-18 levels are associated with nephropathy and atherosclerosis in Japanese patients with type 2 diabetes. *Diabetes Care* 2005; **28**: 2890-2895 [PMID: 16306550 DOI: 10.2337/diacare.28.12.2890]

14 **Letterio JJ**, Roberts AB. Regulation of immune responses by TGF-beta. *Annu Rev Immunol* 1998; **16**: 137-161 [PMID: 9597127 DOI: 10.1146/annurev.immunol.16.1.137]

15 **Rubio-Guerra AF**, Vargas-Robles H, Lozano Nuevo JJ, Escalante-Acosta BA. Correlation between circulating adhesion molecule levels and albuminuria in type-2 diabetic hypertensive patients. *Kidney Blood Press Res* 2009; **32**: 106-109 [PMID: 19342863 DOI: 10.1159/000210554]

16 **Seron D**, Cameron JS, Haskard DO. Expression of VCAM-1 in the normal and diseased kidney. *Nephrol Dial Transplant* 1991; **6**: 917-922 [PMID: 1724689 DOI: 10.1093/ndt/6.12.917]

17 **Lan HY**. Diverse roles of TGF-β/Smads in renal fibrosis and inflammation. *Int J Biol Sci* 2011; **7**: 1056-1067 [PMID: 21927575]

18 **Rubio-Guerra AF**, Cabrera-Miranda LJ, Vargas-Robles H, Maceda-Serrano A, Lozano-Nuevo JJ, Escalante-Acosta BA. Correlation between levels of circulating adipokines and adiponectin/resistin index with carotid intima-media thickness in hypertensive type 2 diabetic patients. *Cardiology* 2013; **125**: 150-153 [PMID: 23736118 DOI: 10.1159/000348651]

19 **Sharma K**, Ramachandrarao S, Qiu G, Usui HK, Zhu Y, Dunn SR, Ouedraogo R, Hough K, McCue P, Chan L, Falkner B, Goldstein BJ. Adiponectin regulates albuminuria and podocyte function in mice. *J Clin Invest* 2008; **118**: 1645-1656 [PMID: 18431508]

20 **Ellington AA**, Malik AR, Klee GG, Turner ST, Rule AD, Mosley TH, Kullo IJ. Association of plasma resistin with glomerular filtration rate and albuminuria in hypertensive adults. *Hypertension* 2007; **50**: 708-714 [PMID: 17785630 DOI: 10.1161/HYPERTENSIONAHA.107.095257]

21 **Lozano-Nuevo JJ**, Estrada-Garcia T, Vargas-Robles H, Escalante-Acosta BA, Rubio-Guerra AF. Correlation between circulating adhesion molecules and resistin levels in hypertensive type-2 diabetic patients. *Inflamm Allergy Drug Targets* 2011; **10**: 27-31 [PMID: 21184654 DOI: 10.2174/187152811794352024]

22 **Rubio-Guerra AF**, Vargas-Robles H, Vargas-Ayala G, Rodriguez-Lopez L, Escalante-Acosta BA. The effect of trandolapril and its fixed-dose combination with verapamil on circulating adhesion molecules levels in hypertensive patients with type 2 diabetes. *Clin Exp Hypertens* 2008; **30**: 682-688 [PMID: 18855271 DOI: 10.1080/10641960802251941]

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MINIREVIEWS

Canagliflozin-current status in the treatment of type 2 diabetes mellitus with focus on clinical trial data

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Abstract

Canagliflozin (CFZ) is a member of new class of glucose lowering agents, sodium-glucose co-transporter (SGLT) inhibitors, which got approval by food and drug administration. It has insulin independent action by blocking the transporter protein SGLT2 in the kidneys, resulting in urinary glucose excretion and reduction in blood glucose levels. In clinical trials, CFZ significantly decreased HbA1c level when administered either as monotherapy or as combined therapy with other anti-diabetic drugs. Intriguingly, it showed additional benefits like weight reduction and lowering of blood pressure. The commonly observed side effects were urinary and genital infections. It has exhibited favorable pharmacokinetic and pharmacodynamic profiles even in patients with renal and hepatic damage. Hence, this review purports to outline CFZ as a newer beneficial drug for type 2 diabetes mellitus.

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Key words: Type 2 diabetes mellitus; Sodium-glucose co-transporter 2; Canagliflozin; Clinical trial; Safety profile

Core tip: This review article focuses upon the current pharmacokinetic, pharmacodynamic and clinical trial data on the newly introduced sodium-glucose co-transporter 2 inhibitor, canagliflozin, for the treatment of type 2 diabetes mellitus. It also discusses briefly about the safety profile and future prospective of canagliflozin.

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by insulin resistance, hyperglycemia and progressive pancreatic β-cell dysfunction. Poorly controlled hyperglycemia leads to irreversible microvascular and macrovascular complications like visual impairment and blindness, kidney failure, peripheral neuropathy, myocardial infarction, stroke and lower limb amputation. In 2012, worldwide > 371 million people suffered from diabetes. Out of which 4.8 million people died due to its complications. This global burden is estimated to increase to 552 million by 2030[1]. This implies that the available drugs for DM are not able to maintain or achieve good glycemic control. Potential adverse events like gastrointestinal disturbances (with biguanides like metformin, α-glucosidase inhibitors like acarbose, glucagon-like peptide-1 agonists like exenatide, amylin agonists like pramlintide), hypoglycemia (with insulin, secretagogues like sulfonylureas and meglitinides), weight gain (with insulin, secretagogues like sulfonylureas and meglitinides, thiazolidinediones like pioglitazone) and risk of cardiovascular disease (with thiazolidinediones like pioglitazone) limit their dosage; and ensuing β-cell failure limits their effectiveness. Current guidelines recommend a target HbA1c value of < 7.0%, with patient-centered

approach allowing some flexibility in terms of the actual target, and treatment with lifestyle changes and drugs for better glycemic control in diabetics. But the target HbA1c is rarely achieved with a single anti-diabetic agent and in only about half of adult patients with diabetes taking combination therapy[2,3]. Hence, there is ongoing hunt for newer efficacious and safer treatment strategies.

Kidney plays a pivotal role in maintaining glucose homeostasis through specialized transporters-sodium-glucose co-transporter (SGLT)1 and SGLT2-present in the proximal convoluted tubule (PCT). Together, they absorb almost all of the glucose filtered in the glomerulus. SGLT1 is a low capacity, high affinity transporter present mostly in small intestine, some in S3 segment of PCT in kidney, and in heart. It is responsible for approximately 10% of glucose reabsorption in the kidney. While SGLT2 is a high capacity, low affinity transporter present almost exclusively in S1 segment of PCT, responsible for approximately 90% of glucose reabsorption[4,5]. But kidney was never the target for treatment of diabetes until phlorizin was discovered. Phlorizin was isolated from the apple trees in 1835 and was initially tested for fever, infectious diseases and malaria. It was noticed that high doses caused glycosuria and chronic administration in dogs caused polydipsia and polyuria with normoglycemia. Subsequent detection of SGLT1 and SGLT2 in kidney, their role in glucose reabsorption and confirmation of inhibitory action of phlorizin on these transporters in animal studies paved way to consider phlorizin in the treatment of type 2 diabetes mellitus (T2DM). However, phlorizin was not clinically developed due to its poor pharmacokinetics and side effects attributed to SGLT1 inhibition such as glucose-galactose malabsorption, dehydration and diarrhea[6,7]. Later on T-1095 was discovered, a derivative of phlorizin which had comparatively better pharmacokinetic profile. Nevertheless, it was discontinued in the Phase-Ⅱ clinical trial[8]. Meanwhile, it was observed that there was upregulation of SGLT2 and increase in maximum tubular transport of glucose in diabetic patients[9]. The underline defect in patients with familial renal glycosuria is also attributable to SGLT2 gene mutation. The patients with gene defect excrete increased amount of glucose in urine and are clinically asymptomatic[10]. These two observations with SGLT2 transporter, *i.e.*, the upregulation of SGLT2 in diabetes and its role in familial renal glycosuria, triggered research that ultimately led to the discovery of specific SGLT2 inhibitors viz. sergliflozin and remogliflozin. Unfortunately, these drugs too exhibited unfavorable pharmacokinetic profile, efficacy and side effect and hence did not progress in clinical trials[11].

Dapagliflozin is the first SGLT2 inhibitor that came to the European market in 2012. Food and drug administration (FDA) approved dapagliflozin on 8th January, 2014[12]. It was initially rejected by FDA due to serious concerns about bladder and breast cancer[13]. Canagliflozin was the first of its kind to get approval from FDA on March 29, 2013. Currently it is in phase-Ⅱ trial for the treatment of obesity in the United States and Europe[14]. Ipragliflozin, empagliflozin and many other SGLT2 inhibitors are under different phases of clinical trials.

This article reviews the available data on the pharmacokinetics, the pharmacodynamics and the therapeutic potential and safety of canagliflozin (CFZ).

SEARCH METHODOLOGY

PubMed, ClinicalTrials.gov and Google scholar databases were used for mining the data. Following Medical subject headings words were used in the above mentioned databases: canagliflozin, canagliflozin and SGLT2, canagliflozin and diabetes, canagliflozin and pharmacokinetics, canagliflozin and pharmacodynamics and canagliflozin and adverse events. Up to date information was included till 31st March 2014.

PHARMACOKINETIC PROPERTIES

When CFZ is taken orally it gets rapidly absorbed from gastrointestinal tract in a dose dependent manner with the dose range of 50-300 mg and mean oral bioavailability of approximately 65%. Median $t_{1/2}$ is 1-2 h and steady state concentration is achieved after 4 to 5 d of daily intake of 100 mg and 300 mg. Maximum plasma concentration is not altered in renal injury. It accumulates in the plasma up to 36% following multiple doses of 100 and 300 mg. The plasma protein binding is 99%, which is constant irrespective of its plasma concentrations or hepatic or renal damage[15,16]. It is metabolized into two inactive O-glucuronide metabolites (M5 and M7). Major O-glucuronidation is by UDP glucuronosyltransferase (UGT)1A9 and UGT2B4, while CYP3A4 mediated oxidative metabolism accounts for only 7%. Single oral radioactive [^{14}C] CFZ to healthy subjects demonstrated 41.5%, 7.0% and 3.2% of administered radioactive dose in feces as CFZ, a hydroxylated metabolite and an O-glucuronide metabolite, respectively. The amount of CFZ excreted in urine in unchanged form is less than 1%, whereas the urine excretion of its metabolites namely M7 is 21%-32% and M5 is 7%-10%. Studies conducted so far have shown no clinically significant effect of age, sex, BMI/weight and race on pharmacokinetics of CFZ[15,16].

PHARMACODYNAMIC PROPERTIES

CFZ primarily inhibits SGLT2 in kidney and is responsible for increased urinary glucose excretion and reduction in blood glucose levels. It also inhibits SGLT1 in intestine and its potency on SGLT1 is 160 times lesser as compared to SGLT2[15,16]. It reduces glucose absorption by 31% in first hour and 20% by next hour of food intake. So, when given before meal, it reduces postprandial glucose excursions[15,17]. This insulin independent action is unique and differentiates CFZ from other available antidiabetic agents. Moreover, there is dose dependent reduction in the renal threshold for glucose excretion (RT_G)

with maximal suppression of RTG from 240 mg/dL to approximately 70-90 mg/dL at the dose of 300 mg. Unlike other oral hypoglycemic drugs, CFZ is tolerated well in mild to moderate hepatic and renal failure patients. However, it is contraindicated in patients with estimated GFR (eGFR) < 30 mL/min per 1.73 m^2, end stage kidney disease and patients on dialysis[15].

DOSAGE AND ADMINISTRATION

The recommended starting dose of CFZ is 100 mg once daily to be taken before the first meal of the day. If patients with eGFR of ⩾ 60 mL/min per 1.73 m^2 tolerate CFZ 100 mg once daily and require additional glycemic control, then dose can be increased to 300 mg once daily. Volume depletion has to be corrected in patients prior to the initiation of CFZ to compensate for CFZ induced increased urination[15].

DRUG INTERACTIONS

UGT inducers (*e.g.*, rifampin, phenytoin, phenobarbital, ritonavir) increase the metabolism of CFZ, thereby reducing active CFZ levels in the blood. Thus, the dose of CFZ may be increased from 100 to 300 mg in such patients. On the other hand, CFZ increases Area Under the Curve for digoxin and hence patients on digoxin treatment should be monitored[15].

THERAPEUTIC POTENTIAL

CFZ has shown promising results in many preclinical and clinical studies of T2DM. A study in Zucker fatty rats and Zucker diabetic fatty rats with CFZ (3-30 mg/kg) decreased renal threshold for glucose and increased urinary glucose excretion (UGE). This resulted in decreased blood glucose, HbA1c, weight gain, dose dependent increased fatty acid metabolism, *de novo* lipogenesis and improved insulin sensitivity in these animals[18].

Table 1 lists the published clinical trials on CFZ use as monotherapy and combined therapy. The CANagliflozin Treatment And Trial Analysis (CANTATA Trials) evaluated CFZ as monotherapy or as an add-on therapy to metformin, metformin and sulphonylurea and metformin and pioglitazone. These trials were randomized; double blind, placebo-or active-controlled with primary endpoint of finding the change in HbA1c at the end of 26 or 52 wk from baseline. In a trial using CFZ as monotherapy, both the doses 100 mg and 300 mg produced a statistically significant decrease in HbA1c ($P < 0.001$), body weight (-2.8% by 100 mg and -3.9% by 300 mg *vs* placebo, $P < 0.001$) as well as systolic blood pressure (-3.7 mmHg by 100 mg and -5.4 mmHg by 300 mg *vs* placebo, $P < 0.001$)[19]. Similar significant results were obtained in combined therapy trials viz. CANTATA-D (Dual therapy trial-CFZ compared with Sitagliptin)[20] and CANTATA-MP (CFZ compared with metformin and pioglitazone)[21].

The CANTATA-SU (CFZ compared with Sulphonylurea) trial established reductions in HbA1c in the glimepiride and CFZ 100 mg groups but greater reductions occurred in CFZ 300 mg group. CFZ 100 mg was reviewed as non-inferior where as CFZ 300 mg group was considered as superior to glimepiride arm. There was greater reduction in body weight, blood pressure (BP) and greater rise in high density lipoprotein (HDL) levels in CFZ group[23]. CANTATA-MSU (CFZ compared with metformin and sulphonylurea) results also demonstrated statistically significant reductions ($P < 0.001$) in HbA1c, fasting blood glucose (FBG) and body weight[24]. In another CANTATA-D2 (Triple therapy trial-CFZ compared with Sitagliptin) trial, at the end of 52 wk, it was showed that CFZ 300 mg was superior to sitagliptin 100 mg when added to sulphonylurea and metformin, in reducing HbA1c, FBG, body weight and systolic blood pressure. There was also significant increase in HDL ($P < 0.001$) in CFZ groups as compared to sitagliptin 100 mg[25].

CANTATA trials have unveiled various interesting clinical observations of CFZ use in the management of T2DM patients. CFZ improved glycemic control without a concomitant increase in the occurrence of hypoglycemia. It lowered RTG but lowering of RTG remained above the hypoglycemic threshold (60-70 mg/dL) and since UGE occurs below the RTG, the incidence as well as risk of hypoglycemia with CFZ was minimal[19,26]. Further, the amplified UGE of 80-120 g/d accounted for net loss of calories (approximately 400 kcal/d) that contributed to the weight loss, which was maintained over the trial period of 52 wk[24,26]. This weight loss was predominantly from loss of fat mass rather than lean body mass[22]. The reversal of glucotoxicity and weight loss together helped to improve beta cell function as indicated in improvement in Homeostasis Model Assessment estimating steady state beta cell function in percentage[19,21,24,26]. The mechanism for increased low-density lipoprotein-C with CFZ is not known, however, improvement in HDL-C and triglycerides was likely to be due to improved glycemic control and weight loss associated with CFZ[19,21,22]. Mild reduction in BP was also observed in the trial participants. This was due to the mild osmotic diuretic response to UGE and natriuretic effect of CFZ[24]. Thus, in nutshell, CFZ can reduce blood glucose levels and has the least risk of producing hypoglycemia as compared to other anti-diabetic agents. In addition, it can also modify the insulin resistance, reduce weight and BP and increase HDL-C. These diverse effects are specific to CFZ and would explain the better outcome with CFZ treated patients as compared to other anti-diabetic agent treatment groups. The CANTATA trials have concluded that CFZ could be taken as an initial drug for T2DM patients whose glycemic control is not achieved with diet and exercise; and also as an effective alternative to sulphonylurea, sitagliptin or pioglitazone in dual therapy with metformin.

CFZ was also studied as an add-on to insulin therapy in a 28-d trial. Participants were T2DM patients not optimally controlled with insulin and receiving up to one oral

Table 1 Summary of clinical trials of canagliflozin

References/trial name (*n* = sample size)	Study population and duration of the study	Study drugs	Change in HbA1c from the baseline (in percent)	Change in FBG from baseline	Change in body weight from baseline	Other parameters (least square mean change)
Stenlöf *et al*[19] (*n* = 584)	T2DM patients on diet and exercise with inadequate glycemic control Duration of the study = 26 wk	CFZ = 100 mg/300 mg OD *vs* PL	-0.77% to -1.03% (CFZ 100 mg/300 mg, $P < 0.001$ *vs* PL)	-27 mg/dL to -35 mg/dL (CFZ 100 mg/300 mg, $P < 0.001$ *vs* PL)	% Body weight reduction -2.8% to -3.9% (CFZ 100 mg/300 mg, $P < 0.001$ *vs* PL)	ΔPPBG = -43 to -59 mg/dL (CFZ 100 mg/300 mg) ΔSBP = -3.3 to -5.0 mmHg (CFZ 100 mg/300 mg, $P < 0.001$ *vs* PL) ΔDBP = -1.7 to -2.1 mmHg (CFZ 100 mg/300 mg *vs* PL) ΔHDL = CFZ 100 mg = 11.2% ($P < 0.001$ *vs* PL) CFZ 300 mg = 10.6% ($P < 0.01$ *vs* PL) ΔLDL = 2.9% to 7.1% (CFZ 100 mg/300 mg *vs* PL) ΔTG = 2.5% to -2.3% (CFZ 100 mg/300 mg *vs* PL) HOMA-%B = 9.9% to 20.3% (CFZ 100 mg/300 mg *vs* PL)
CANTATA-D[20] (*n* = 1284)	26-wk extension study T2DM patients with inadequate glycemic control on protocol specified MET-IR monotherapy with HbA1c: 7.0% to 10.5%, FBG < 270 mg/dL	CFZ = 100 mg/300 mg OD + MET-IR *vs* PL + MET-IR for first 26 wk	At the end of 26 wk -0.79% to -0.94% (CFZ 100 mg/300 mg, $P < 0.001$ *vs* PL)	At the end of 26 wk -27.3 mg/dL to -37.8 mg/dL (CFZ 100 mg/300 mg, $P < 0.001$ *vs* PL)	At the end of 26 wk % Body weight reduction -3.7% to -4.2% (CFZ 100 mg/300 mg, $P < 0.001$ *vs* PL)	At the end of 26 wk ΔPPBG = -47.9 mg/dL to -57.1 mg/dL (CFZ 100 mg/300 mg, $P < 0.001$ *vs* PL) SITA = -49.3 mg/dL ΔSBP = -3.84 mmHg to -5.06 mmHg (CFZ 100 mg/300 mg, $P < 0.001$ *vs* PL), SITA = -1.83 mmHg ΔTG = CFZ 100 mg = 1.6%, $P = 0.7$ *vs* PL, CFZ 300 mg = -1.4%, $P = 0.2$ *vs* PL, SITA = 1.0% ΔHDL = 10.4% to 12.1% (CFZ 100 mg/300 mg, $P < 0.001$ *vs* PL), SITA = 5%
		CFZ = 100 mg/300 mg OD + MET-IR *vs* SITA 100 mg + MET-IR for next 26 wk	SITA = -0.82%	SITA = -20.2 mg/dL	SITA = -1.2%	
			At the end of 52 wk -0.73% to -0.88% (CFZ 100 mg/300 mg)	At the end of 52 wk -26.2 mg/dL to -35.2 mg/dL (CFZ 100 mg/300 mg, $P < 0.001$ *vs* SITA)	At the end of 52 wk -3.8% to -4.2% (CFZ 100 mg/300 mg, $P < 0.001$ *vs* SITA)	At the end of 52 wk ΔSBP = -3.53 mmHg to -4.65 mmHg (CFZ 100 mg/300 mg, $P < 0.001$ *vs* SITA) SITA = -0.66 mmHg ΔTG = CFZ 100 mg = 1.9%, $P = 0.46$ *vs* SITA CFZ 300 mg = 2.7%, $P = 0.32$ *vs* SITA SITA = -0.4% ΔHDL = 11.2% to 13.3% (CFZ 100 mg/300 mg, $P < 0.001$ *vs* SITA), SITA = 6.0%
			SITA = -0.73%	SITA = -17.7 mg/dL	SITA = -1.3%	
Guthrie *et al*[21] Forst *et al*[22] CANTATA-MP (*n* = 344)	26-wk extension study T2DM patients currently treated with PPAR gamma agent (PIO or ROSI) and MET with HbA1c: 7%-10.5% and FBG < 270 mg/dL	CFZ = 100 mg/300 mg OD + MET + PIO *vs* PL + MET + PIO for first 26 wk CFZ = 100 mg/300 mg OD + MET + PIO *vs* SITA 100 mg + MET + PIO for next 26 wk	At the end of 26 wk -0.89% to -1.03% (CFZ 100 mg/300 mg, $P < 0.001$ *vs* PL)	At the end of 26 wk -26.8 mg/dL to -33.2 mg/dL (CFZ 100 mg/300 mg, $P < 0.001$ *vs* PL)	At the end of 26 wk -2.8% to -3.8% (CFZ 100 mg/300 mg, $P < 0.001$ *vs* PL)	At the end of 26 wk ΔSBP = CFZ 100 mg = -5.30 mmHg ($P = 0.005$ *vs* PL) CFZ 300 mg = -4.70 mmHg ($P = 0.016$ *vs* PL) ΔTG = CFZ 100 mg = 3.2% ($P = 0.034$ *vs* PL) CFZ 300 mg = -1.7% ($P = 0.003$ *vs* PL) ΔHDL = CFZ 100 mg = 7.2% ($P = 0.01$ *vs* PL) CFZ 300 mg = 8.9% ($P < 0.001$ *vs* PL) HOMA-%B = 15.19% to 18.14% (CFZ 100 mg/300 mg, $P < 0.01$ *vs* PL)
			At the end of 52 wk -0.92% to -1.03% (CFZ 100 mg/300 mg)	At the end of 52 wk -26.7 mg/dL to -31.5 mg/dL (CFZ 100 mg/300 mg)	At the end of 52 wk -2.7% to -3.7% (CFZ 100 mg/300 mg)	At the end of 52 wk ΔSBP = -3.4 to -3.7 mmHg (CFZ 100 mg/300 mg) ΔDBP = -2.5 to -2.7 mmHg (CFZ 100 mg/300 mg) ΔHDL = CFZ 100 mg = 7.0% CFZ 300 mg = 11.4% ΔLDL = 10.9% to 14.3% (CFZ 100 mg/300 mg) ΔTG = 4.7% to -0.6% (CFZ 100 mg/300 mg)

Cefalu *et al*[23] CANTATA-SU (*n* = 1450)	T2DM patients with HbA1c: 7%-9.5% on stable MET therapy ⩾ 1500 mg/d, BMI = 22-45kg/m^2, FBG ⩽ 270mg/dL Duration of the study = 52 wk	CFZ = 100 mg/300mg OD + MET *vs* GLIM 6 mg/8 mg OD + MET	-0.82% to -0.93% (CFZ 100 mg/300 mg) GLIM = -0.81%	-1.35 mmol/L to -1.52 mmol/L (CFZ 100 mg/300 mg) GLIM = -1.02 mmol/L	-4.2% to -4.7% (CFZ 100 mg/300 mg, *P* < 0.001 *vs* GLIM) GLIM = 1%	ΔSBP = -3.3 to -4.6 mmHg (CFZ 100 mg/300 mg) GLIM = 0.2 mmHg ΔDBP = -1.8 to -2.5 mmHg (CFZ 100 mg/300 mg) GLIM = -0.1 mmHg ΔTG = CFZ 100 mg = -3.7% CFZ 300 mg = 2.3% GLIM = 9.5% ΔHDL = CFZ 100 mg = 7.9% CFZ 300 mg = 9.0% GLIM = 0.3% ΔLDL = 9.6% to 14.1% (CFZ 100 mg/300 mg) GLIM = 5%
Wilding *et al*[24] CANTATA-MSU (*n* = 469)	26-wk extension study T2DM patients currently treated with MET and SU with HbA1c: 7%-10.5% and FBG < 270 mg/dL	CFZ = 100 mg/300 mg OD + MET + SU *vs* PL + MET + SU	At the end of 26 wk -0.85% to -1.06% (CFZ 100 mg/300 mg, *P* < 0.001 *vs* PL)	At the end of 26 wk -18.2 mg/dL to -30.5 mg/dL (CFZ 100 mg/300 mg, *P* < 0.001 *vs* PL)	At the end of 26 wk -2.1% to -2.6% (CFZ 100 mg/300 mg, *P* < 0.001 *vs* PL)	At the end of 26 wk ΔSBP = CFZ 100 mg = -4.89 mmHg (*P* = 0.07 *vs* PL) CFZ 300 mg = -4.27 mmHg (*P* = 0.2 *vs* PL) ΔTG = CFZ 100 mg = 5.4% (*P* = 0.256 *vs* PL) CFZ 300 mg = 8.5% (*P* = 0.57 *vs* PL) ΔHDL = CFZ 100 mg = 5.7% (*P* = 0.153 *vs* PL) CFZ 300 mg = 6.5% (*P* = 0.056 *vs* PL)
Schernthaner *et al*[25] CANTATA-D2 (*n* = 755)	T2DM patients currently treated with MET and SU with HbA1c: 7%-10.5% and FBG < 300 mg/dL Duration of the study = 52 wk	CFZ = 300 mg OD + MET + SU *vs* SITA = 100 mg OD + MET + SU	CFZ 300 mg = -1.03% (*P* < 0.001 *vs* SITA) SITA = -0.66%	CFZ 300 mg = -29.9 mg/dL (*P* < 0.001 *vs* SITA) SITA = -5.85 mg/dL	CFZ 300 mg = -2.5% (*P* < 0.001 *vs* SITA) SITA = 0.3%	ΔSBP = CFZ 300 mg = -5.06 mmHg (*P* < 0.001 *vs* SITA) SITA = 0.85 mmHg ΔTG = CFZ 300 mg = 9.6% (*P* = 0.554 *vs* SITA) SITA = 11.9% ΔHDL = CFZ 300 mg = 7.6% (*P* < 0.001 *vs* SITA) SITA = 0.6%
Devineni *et al*[26] (*n* = 29)	T2DM patients not optimally controlled on insulin and up to one oral AHA with BMI: 25-45 kg/m^2, FBG: 3.3-5.5 mmol/L, HbA1c: 7%-10.5% and serum creatinine: < 132.6 mmol/L for males and < 123.8 mmol/L for women Duration of the study = 28 d	CFZ 100 mg OD/300 mg bid + Insulin + upto one AHA *vs* PL + insulin + upto one AHA	-0.73% to -0.92% (CFZ 100 mg/300 mg)	-2.11 mmol/L to -2.35 mmol/L (CFZ 100 mg/300 mg)	-0.73 kg to -1.19 kg (CFZ 100 mg/300 mg)	ΔUGE = 71.9 g/d to 129.2 g/d
Rosenstock *et al*[27] (*n* = 451)	T2DM patients under stable MET monotherapy (⩾ 1500 mg/d) with HbA1c: 7%-10%, BMI: 24-45 kg/m^2, serum creatinine: < 1.5mg/dL for males and < 1.4 mg/dL for females Duration of the study = 12 wk	CFZ = 50/100/200/300 mg OD or 300 mg bid + MET *vs* PL + MET *vs* SITA 100 mg OD + MET	CFZ 50 mg = -0.79% 100 mg = -0.76% 200 mg = -0.70% 300 mg = -0.92% 300 mg bid = -0.95% SITA = -0.74%	CFZ 50 mg = -16.2 mg/dL 100 mg = -25.2 mg/dL 200 mg = -27.0 mg/dL 300 mg = -25.2 mg/dL 300 mg bid = -3.4 mg/dL SITA = -12.6 mg/dL	CFZ 50 mg = -2.3% 100 mg = -2.60% 200 mg = -2.70% 300 mg = -3.40% 300 mg bid = -3.4% SITA = -0.60%	ΔSBP = -3.3 to -5 mmHg (CFZ 100 mg/300 mg) SITA = -0.8 mmHg ΔDBP = -1.7 to -2.1 mmHg (CFZ 100 mg/300 mg) SITA = -0.6 mmHg ΔTG = CFZ 100 mg = 2.5% CFZ 300 mg = -2.3% ΔHDL = CFZ 100 mg = 11.2% CFZ 300 mg = 10.6% ΔLDL = 2.9% to 7.1% (CFZ 100 mg/300 mg) HOMA-%B = CFZ 50-300 mg OD = 6% to 18% CFZ 300 mg bid = 16% SITA = 10%

ClinicalTrials.gov identifier: NCT01064414[28] (*n* = 272)	26-wk extension study T2DM patients with or without AHA, on regular diet and exercise with moderate renal impairment	CFZ = 100 mg/300 mg OD with or without AHA *vs* PL with or without AHA	At the end of 26 wk -0.33% to -0.44% (CFZ 100 mg, *P* = 0.01 *vs* PL, CFZ 300 mg, *P* < 0.001 *vs* PL)	At the end of 26 wk CFZ 100 mg = -14.9 mg/dL, *P* = 0.02 CFZ 300 mg = -11.7 mg/dL, *P* = 0.06	Information was not available	Information was not available

OD: Once daily; FBG: Fasting blood glucose; PL: Placebo; CFZ: Canagliflozin; ΔSBP: Change in systolic blood pressure from baseline; ΔDBP: Change in diastolic blood pressure from baseline; ΔHDL: Change in blood HDL level from baseline; ΔLDL: Change in blood LDL level from baseline; ΔTG: Change in blood triglycerides level from baseline; ΔPPBG: Change in postprandial blood glucose from baseline; MET: Metformin; SU: Sulphonylurea; SITA: Sitagliptin; PIO: Pioglitazone; ROSI: Rosiglitazone; AHA: Antihyperglycemic agent; IR: Immediate release; GLIM: Glimepride; HOMA-%B: Homeostasis Model Assessment estimating steady state beta cell function in percentage; LDL: Low-density lipoprotein; T2DM: Type 2 diabetes mellitus; PPAR: Peroxisome proliferator activated receptor.

antihyperglycemic agent. Both the CFZ doses (100 mg and 300 mg) showed greater reduction in HbA1c, body weight and FBG[26].

The effects of various doses of CFZ (50, 100, 200, 300 mg OD and 300 mg BD) have also been assessed in a 12-wk trial in T2DM patients under stable metformin therapy. CFZ demonstrated greater reduction in FBG and body weight at all doses as compared to sitagliptin[27].

Three studies conducted trials in special patient population. One study was on adults with T2DM aged 55 to 80 years, not controlled on diet and exercise together with an antihyperglycemic agent. This trial showed that CFZ is equally effective in this age group[15]. In the second study, CFZ showed significant reduction in HbA1c in T2DM patients with or without an antihyperglycemic agent, on regular diet and exercise with moderate renal impairment[29]. The third trial done on T2DM patients with stage 3 chronic kidney disease (CKD) established the safety and efficacy of CFZ in these patients as well[28].

SAFETY PROFILE

Overall, CFZ is well tolerated. The distinctive concern is about increased risk of genital mycotic infections and urinary tract infections (UTI). The data from the four pooled 26-wk placebo-controlled trials including monotherapy trial and three add-on combination trials with metformin, metformin and sulfonylurea or metformin and pioglitazone; demonstrated female genital mycotic infections in 3.2% of patients in placebo, 10.4% in CFZ 100 mg and 11.4% of patients in CFZ 300 mg groups. The incidence of genital mycotic infection was less in males with rates of 0.6% in placebo, 4.2% in CFZ 100 mg and 3.7% in CFZ 300 mg groups. UTI presented at the rate of 4% in the placebo, 5.9% in 100 mg dose and 4.3% in the 300 mg dose groups[15].

Other common adverse events reported were increased urination, vulvovaginal pruritus, thirst, constipation and nausea. The risk of hypoglycemia in patients with CFZ was generally low. Volume depletion-related adverse reactions such as dizziness, hypotension and dehydration were higher in elderly patients, 65 years or older, particularly with the 300 mg daily dose, in comparison to younger patients. There were mild and transient changes in eGFR, albumin-creatinine ratio and blood urea nitrogen in early phase of the study in stage 3 CKD patients. However, 26-wk treatment caused return of these parameters to baseline; there was also an increase in serum potassium and magnesium in these patients[15,29]. Some studies showed increase in LDL-C and hematocrit[15]. These short-term studies have shown no significant changes in vital signs or electrocardiogram finding with CFZ use. A long term prospective study to evaluate the efficacy and adverse effect profile of CFZ is already underway[30]. The summary of adverse events of CFZ is depicted in Table 2.

CONCLUSION

Clinical trial data for CFZ reveal that its glucose lowering efficacy is superior to usual gold standard drugs with the added benefit of weight loss. FDA has already approved CFZ monotherapy in adjuvant to diet and exercise. So far, none of the serious concerns which surround dapagliflozin are seen in CFZ trials. Furthermore, its insulin independent action is an important advantage as this essentially means that its glucose-lowering efficacy should not decrease significantly with progression of diabetes. CFZ is also compatible with other anti-diabetic therapies, including insulin, and might therefore be of value at any stage in the natural history of T2DM. CFZ with its multi dimensional properties can be beneficial in the disease cluster of obesity, hypertension and diabetes. Further, there is a low propensity to cause hypoglycemia in patients as glucose is reab-

Table 2 Summary of adverse events observed in the canagliflozin clinical trials

S. No	ClinicalTrials.gov identifier	Adverse events	Ref.
1	NCT01081834	Increased incidence of AEs in CFZ groups Serious AEs and AE related discontinuations similar in all groups Increased incidence of UTI, genital mycotic infections and osmotic diuresis related AEs in CFZ groups Moderate increase in BUN, serum creatinine and decrease in serum uric acid	Stenlöf *et al*[19]
2	NCT01106677	AEs similar across all groups Higher incidence of pollakiuria in CFZ groups -5.71% with 100 mg CFZ and 2.72% with 300 mg CFZ *vs* 0.55% of PL	[20]
3	NCT01106690	Vulvovaginal mycotic infections: 2.65% to 5.26% *vs* 0% of placebo Pollakiuria: 6.14% to 9.42% *vs* 0.87% of placebo Increased rate of hypoglycemic event with CFZ 300 mg (5.26% *vs* 1.74% of PL)	Guthrie *et al*[21]
4	NCT00968812	Osmotic diuresis related AEs in 3% of CFZ groups as compared to < 1% in placebo groups Genital infections and increase in LDL cholesterol more in CFZ groups	Cefalu *et al*[23]
5	NCT01106625	Superficial genital mycotic infection: 16.0% to 21.0% *vs* 5% in women and 3.4% to 6.6% *vs* 1.3% in men More subjects treated with CFZ had ⩾ 1 hypoglycemic episodes	Wilding *et al*[24]
6	NCT01137812	Genital mycotic infections: 9.2% of CFZ 300 mg *vs* 0.5% of SITA Osmotic diuresis related AEs: 2.4% of CFZ 300 mg *vs* 1.3% of SITA Higher incidence of increased TG in CFZ groups	Schernthaner *et al*[25]
7	Not available	Similar rate of AEs and discontinuations across all groups No serious AEs	Devineni *et al*[26]
8	NCT00642278	Non dose dependent increase in incidence of genital infections (3%-8% *vs* 2% of SITA) and UTI (3%-9% *vs* 2% of SITA) in CFZ groups Low incidence of hypoglycemia Small increase in LDL cholesterol in CFZ groups	Rosenstock *et al*[27]
9	NCT01064414	AEs similar across all groups Increased incidence of hypoglycemic events in CFZ groups -14.44% with 100 mg CFZ and 11.24% with 300 mg CFZ *vs* 4.44% of PL	[28]

AEs: Adverse events; CFZ: Canagliflozin; UTI: Urinary tract infections; SITA: Sitagliptin; LDL: Low-density lipoprotein; PL: Placebo; TG: Triglycerides; BUN: Blood urea nitrogen.

sorbed by SGLT1 in kidney. In addition to the reported side effects of CFZ like UTI, genital mycotic infections, volume depletion and hypotension, the high cost of CFZ may prove to be a limiting factor in its wide spread use. However, for the time being CFZ has been proven to be safe and well tolerated and it is for the further long term studies to establish it more firmly as a major breakthrough in the clinical armamentarium for patients with diabetes.

REFERENCES

1 Global diabetes plan at a glance 2011-2021. International diabetes federation. Available from: URL: http: //www.idf.org/sites/default/files/Global_Diabetes_Plan_Final.pdf

2 **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]

3 **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]

4 **Kanai Y**, Lee WS, You G, Brown D, Hediger MA. The human kidney low affinity Na+/glucose cotransporter SGLT2. Delineation of the major renal reabsorptive mechanism for D-glucose. *J Clin Invest* 1994; **93**: 397-404 [PMID: 8282810 DOI: 10.1172/JCI116972]

5 **Bailey CJ**. Renal glucose reabsorption inhibitors to treat diabetes. *Trends Pharmacol Sci* 2011; **32**: 63-71 [PMID: 21211857 DOI: 10.1016/j.tips.2010.11.011]

6 **Mackenzie GM**. An experimental study of blood glycolysis. the effects of thyroid and adrenal extracts and phlorhizin on glycolysis in vitro. *J Exp Med* 1915; **22**: 757-765 [PMID: 19867956 DOI: 10.1084/jem.22.6.757]

7 **Ehrenkranz JR**, Lewis NG, Kahn CR, Roth J. Phlorizin: a review. *Diabetes Metab Res Rev* 2005; **21**: 31-38 [PMID: 15624123 DOI: 10.1002/dmrr.532]

8 **Isaji M**. SGLT2 inhibitors: molecular design and potential differences in effect. *Kidney Int Suppl* 2011; (**120**): S14-S19 [PMID: 21358697 DOI: 10.1038/ki.2010.511]

9 **Freitas HS**, Anhê GF, Melo KF, Okamoto MM, Oliveira-Souza M, Bordin S, Machado UF. Na(+) -glucose transporter-2 messenger ribonucleic acid expression in kidney of diabetic rats correlates with glycemic levels: involvement of hepatocyte nuclear factor-1alpha expression and activity. *Endocrinology* 2008; **149**: 717-724 [PMID: 17962340 DOI: 10.1210/en.2007-1088]

10 **Santer R**, Kinner M, Lassen CL, Schneppenheim R, Eggert P, Bald M, Brodehl J, Daschner M, Ehrich JH, Kemper M, Li Volti S, Neuhaus T, Skovby F, Swift PG, Schaub J, Klaerke D. Molecular analysis of the SGLT2 gene in patients with renal glucosuria. *J Am Soc Nephrol* 2003; **14**: 2873-2882 [PMID: 14569097 DOI: 10.1097/01.ASN.0000092790.89332.D2]

11 **Washburn WN**. Evolution of sodium glucose co-transporter 2 inhibitors as anti-diabetic agents. *Expert Opin Ther Pat* 2009; **19**: 1485-1499 [PMID: 19852718 DOI: 10.1517/13543770903337828]

12 FDA approves Farxiga to treat type 2 diabetes. Available from: URL: http: //www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm380829.htm

13 **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]
14 **Elkinson S**, Scott LJ. Canagliflozin: first global approval. *Drugs* 2013; **73**: 979-988 [PMID: 23729000 DOI: 10.1007/s40265-013-0064-9]
15 **Janssen Pharmaceuticals Inc**. Invokana™ (canagliflozin) tablets, for oral use: US prescribing information. Available from: URL: http: //www.janssenmd.com/pdf/invokana/PI-INVOKANA.pdf
16 **Janssen Pharmaceuticals Inc**. Endocrinologic and Metabolic Drugs Advisory Committee: Canagliflozin as an Adjunctive Treatment to Diet and Exercise Alone or Co-administered with Other Antihyperglycemic Agents to Improve Glycemic Control in Adults with Type 2 Diabetes Mellitus. Available from: URL: http: //www.fda.gov/downloads/AdvisoryCommittees/.../UCM334551.pdf
17 **Polidori D**, Sha S, Mudaliar S, Ciaraldi TP, Ghosh A, Vaccaro N, Farrell K, Rothenberg P, Henry RR. Canagliflozin lowers postprandial glucose and insulin by delaying intestinal glucose absorption in addition to increasing urinary glucose excretion: results of a randomized, placebo-controlled study. *Diabetes Care* 2013; **36**: 2154-2161 [PMID: 23412078 DOI: 10.2337/dc12-2391]
18 **Liang Y**, Arakawa K, Ueta K, Matsushita Y, Kuriyama C, Martin T, Du F, Liu Y, Xu J, Conway B, Conway J, Polidori D, Ways K, Demarest K. Effect of canagliflozin on renal threshold for glucose, glycemia, and body weight in normal and diabetic animal models. *PLoS One* 2012; **7**: e30555 [PMID: 22355316 DOI: 10.1371/journal.pone.0030555]
19 **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]
20 **Janssen Research & Development, LLC**. The CANTATA-D Trial (CANagliflozin Treatment and Trial Analysis-DPP-4 Inhibitor Comparator Trial). Available from: URL: http: //clinicaltrials.gov/ct2/show/record/NCT01106677?term=CANTATA-D&rank= 1& sect=X0125
21 **Guthrie R**, Goldenberg R, Vijapurkar U, Yee J, Meininger G, Stein P. Efficacy and safety of canagliflozin in subjects with type 2 diabetes mellitus on metformin and pioglitazone [abstract]. 4th World Congress on Controversies to Consensus in Diabetes, Obesity and Hypertension; 8-11 November 2012; Barcelona, Spain
22 **Forst T**, Guthrie R, Goldenberg R, Yee J, Vijapurkar U, Meininger G, Stein P. Efficacy and safety of canagliflozin over 52 weeks in patients with type 2 diabetes on background metformin and pioglitazone. *Diabetes Obes Metab* 2014; **16**: 467-477 [PMID: 24528605 DOI: 10.1111/dom.12273]
23 **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: 10.1016/S0140-6736(13)60683-2]
24 **Wilding JP**, Mathieu C, Vercruysse F, Usiskin K, Deng L, Canovatchel W. Canagliflozin, a sodium glucose co-transporter 2 inhibitor, improves glycaemia in subjects with type 2 diabetes inadequately controlled with metformin plus sulphonylurea [abstract no. 766]. *Diabetologia* 2012; **55** Suppl 1: S315-S316
25 **Schernthaner G**, Gross JL, Rosenstock J, Guarisco M, Fu M, Yee J, Kawaguchi M, Canovatchel W, Meininger G. Canagliflozin compared with sitagliptin for patients with type 2 diabetes who do not have adequate glycemic control with metformin plus sulfonylurea: a 52-week randomized trial. *Diabetes Care* 2013; **36**: 2508-2515 [PMID: 23564919 DOI: 10.2337/dc12-2491]
26 **Devineni D**, Morrow L, Hompesch M, Skee D, Vandebosch A, Murphy J, Ways K, Schwartz S. Canagliflozin improves glycaemic control over 28 days in subjects with type 2 diabetes not optimally controlled on insulin. *Diabetes Obes Metab* 2012; **14**: 539-545 [PMID: 22226086 DOI: 10.1111/j.1463-1326.2012.01558.x]
27 **Rosenstock J**, Aggarwal N, Polidori D, Zhao Y, Arbit D, Usiskin K, Capuano G, Canovatchel W. Dose-ranging effects of canagliflozin, a sodium-glucose cotransporter 2 inhibitor, as add-on to metformin in subjects with type 2 diabetes. *Diabetes Care* 2012; **35**: 1232-1238 [PMID: 22492586 DOI: 10.2337/dc11-1926]
28 **Janssen Research & Development, LLC**. An Efficacy, Safety, and Tolerability Study of Canagliflozin in Patients With Type 2 Diabetes Mellitus Who Have Moderate Renal Impairment. Available from: URL: http: // clinicaltrials.gov/ct2/show/results/NCT01064414?term=NCT01064414&rank=1§=X4305#othr
29 **Yale JF**, Bakris G, Xi L, Figueroa K, Wajs E, Usiskin K, Meininger G. Canagliflozin, a sodium glucose co-transporter 2 (SGLT2) inhibitor, improves glycaemic control and is well tolerated in type 2 diabetes mellitus (T2DM) subjects with moderate renal impairment [abstract no. 759]. *Diabetologia* 2012; **55** Suppl 1: S312
30 **Janssen Research & Development, LLC**. CANVAS - CANagliflozin cardioVascular Assessment Study. Available from: URL: http: //clinicaltrials.gov/show/NCT01032629

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RANDOMIZED CONTROLLED TRIAL

Impact of chronic disease self-management programs on type 2 diabetes management in primary care

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Abstract

AIM: To assess the effectiveness of the Chronic Disease Self-Management Program (CDSMP) on glycated hemoglobin A1c (HbA1c) and selected self-reported measures.

METHODS: We compared patients who received a diabetes self-care behavioral intervention, the CDSMP developed at the Stanford University, with controls who received usual care on their HbA1c and selected self-reported measures, including diabetes self-care activities, health-related quality of life (HRQOL), pain and fatigue. The subjects were a subset of participants enrolled in a randomized controlled trial that took place at seven regional clinics of a university-affiliated integrated healthcare system of a multi-specialty group practice between January 2009 and June 2011. The primary outcome was change in HbA1c from randomization to 12 mo. Data were analyzed using multilevel statistical models and linear mixed models to provide unbiased estimates of intervention effects.

RESULTS: Demographic and baseline clinical characteristics were generally comparable between the two groups. The average baseline HbA1c values in the CDSMP and control groups were 9.4% and 9.2%, respectively. Significant reductions in HbA1c were seen at 12 mo for the two groups, with adjusted changes around 0.6% ($P < 0.0001$), but the reductions did not differ significantly between the two groups ($P = 0.885$). Few significant differences were observed in participants' diabetes self-care activities. No significant differences were observed in the participants' HRQOL, pain, or fatigue measures.

CONCLUSION: The CDSMP intervention may not lower HbA1c any better than good routine care in an integrated healthcare system. More research is needed to understand the benefits of self-management programs in primary care in different settings and populations.

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Key words: Type 2 diabetes; Self-management; Chronic Disease Self-Management Program; Glycemic control; Glycated hemoglobin; Chronic disease

Core tip: Diabetes is a serious chronic disease. One of the most studied evidence-based behavioral or self-care programs targeting chronic conditions including diabe-

tes is the Stanford Chronic Disease Self-Management Program (CDSMP). Although the CDSMP has been studied extensively, its impact on glycemic control has not been thoroughly evaluated in a randomized controlled trial to date. To the best of our knowledge, this is the first study to evaluate the effectiveness of the CDSMP in a randomized controlled trial. Our finding that the CDSMP intervention may not lower hemoglobin A1c any better than good routine care in an integrated healthcare system calls for further research.

Forjuoh SN, Ory MG, Jiang L, Vuong AM, Bolin JN. Impact of chronic disease self-management programs on type 2 diabetes management in primary care. *World J Diabetes* 2014; 5(3): 407-414 Available from: URL: http://www.wjgnet.com/1948-9358/full/v5/i3/407.htm DOI: http://dx.doi.org/10.4239/wjd.v5.i3.407

INTRODUCTION

Diabetes is a serious chronic condition affecting millions of people worldwide. According to estimates by the World Health Organization, about 350 million people have diabetes globally[1]. Diabetes has a severe and significant health and economic impact on all nations. It is the 6th leading cause of death in Canada and the 7th leading cause of death in the United States, costing an estimated $174 billion[2,3]. The bulk of this cost is attributable to the serious long-term complications associated with the condition including limb amputations, blindness, coronary health disease, stroke, and kidney disease[3]. Type 2 diabetes accounts for 90%-95% of all diabetes[3]. Although type 2 diabetes is more prevalent among people aged 40 years or older, the prevalence among younger populations is increasing dramatically because of the rise in obesity and physical inactivity in children and the youth[4].

Supportive programs to enhance patient self-care have been touted as a pre-requisite to diabetes management in spite of differences in individual needs to cope with this debilitating condition[5]. The traditional didactic models of care that involved teaching patients to improve the knowledge of their health condition are giving way to the current models that focus on behavioral or self-care approaches aimed at providing patients with the skills and strategies to promote and change their behavior[6]. In fact, several national organizations including the American Diabetes Association and the American Association of Diabetes Educators consider self-care an essential component of effective diabetes management[7-9].

One of the most studied evidence-based behavioral or self-care programs targeting chronic conditions is the Chronic Disease Self-Management Program (CDSMP). Developed at the Stanford University, the program offers the potential to improve overall health of individuals with chronic conditions, while preventing further decline in their general health status[10-12]. Designed as a 6-wk, community-based self-care education program, CDSMP focuses on assisting participants to gain confidence or self-efficacy and acquire skills to better manage their chronic conditions. It is taught by trained leaders using a structured protocol.

The CDSMP has been found to be highly effective in improving general health and lowering hospitalization rates[10]. It has therefore been implemented worldwide for several chronic conditions such as heart disease, lung disease, arthritis, and diabetes as well as evaluated in various settings including the United States, Canada, United Kingdom, Australia, New Zealand, Bangladesh, China, Hong Kong, and The Netherlands[13-20]. While the original CDSMP validation study found improvements in general health status, health behaviors, and healthcare utilization[10], the findings of more recent studies from a variety of self-management programs have been inconsistent[5,21-27]. A recent literature review of randomized controlled trials comparing self-management support interventions for general chronic diseases *vs* usual care revealed mixed results. While positive findings were found regarding self-efficacy, less positive ones were found for quality-of-life measures[5]. Also although the CDSMP has been studied extensively, its impact on glycemic control has not been thoroughly assessed. In particular, its effectiveness on glycemic control has not been evaluated in a randomized controlled trial in the United States to date. A recent study concluded that the CDSMP is a useful and appropriate program for lowering glycated hemoglobin A1c (HbA1c) among those out of control[28]. However, this was a longitudinal study with no comparison group. Another related study found the CDSMP to improve lifestyle behaviors among patients with type 2 diabetes[23,29]. But again this was a single-group design.

The aim of this study was to assess the effectiveness of the CDSMP on glycemic control and selected self-reported measures among patients with type 2 diabetes in a large integrated healthcare organization in central Texas that serves large racially/ethnically diverse populations.

MATERIALS AND METHODS

Design

This study was a comparison of one intervention arm, the CDSMP, and the control arm from an open-label, 4-arm randomized controlled trial that was designed to evaluate the effectiveness of two different type 2 diabetes mellitus (T2DM) self-care interventions (implemented singly and in combination) on glycemic control. Designed with the acknowledgment that both patients and researchers would be aware of the random assignment, the study protocol consisted of screening potential subjects for eligibility, randomizing them to one of four study arms, and following them over a 24-mo period. However, the primary end-point was change in HbA1c from randomization/baseline to 12 mo of follow-up. The current study reported here focuses on participants in two of the four original study arms.

The study protocol was approved by the Institutional Review Boards (IRB) of Scott and White Healthcare System and Texas A and M Health Science Center. All quali-

fied participants accepted the conditions of the study and gave informed written consent at enrollment/orientation. Enrollment occurred between January 2009 and June 2011 and data collection was completed in July 2012. We adhered to the CONSORT protocol[30] and registered the trial with clinicaltrials.gov (NCT01221090).

Setting, participants, and recruitment

Participants represent a subset of subjects that were recruited from seven participating clinics of a large integrated healthcare system, a university-affiliated, multispecialty group practice associated with a 250000-member Health Maintenance Organization in central Texas. Potential participants were identified through electronic medical records if they: (1) had a diagnosis of T2DM; (2) were ≥ 18 years; (3) had a lab assessed HbA1c value ≥ 7.5% (≥ 58 mmol/mol) within the last six months; and (4) were able to communicate in English. Subjects were excluded if they: (1) had documented reports of alcoholism or drug abuse; (2) were pregnant or planning to become pregnant within 12 mo; or (3) were unwilling to sign an informed consent. Recruitment was solicited by physicians within the seven clinics who agreed to invite their patients to participate in the study.

Physicians were provided with IRB approved invitation-to-participate letters and a list of their T2DM patients meeting the threshold HbA1c level at their last visit. Contact was initiated with potential subjects through physician-sent letters, describing the study and requesting a completed screening enrollment card if interested. Subjects who returned a screening enrollment card were contacted by project coordinators, who provided additional information and screened them to determine eligibility. To verify the inclusion and exclusion criteria, subject permission was obtained to review their medical records. Other recruitment strategies included oral referrals by physicians and patient educators and posting messages in waiting areas of study clinics.

Lab assessments were continuously monitored at each phase of the study recruitment to ensure that enrolled participants had HbA1c values ≥ 7.5% (≥ 58 mmol/mol) within the last six months since individuals who previously met this criterion may no longer fulfill that requirement at orientation. A follow-up telephone interview was conducted to determine participation interest. Lab results were screened to ensure that the participant met qualifying HbA1c and if needed, tests were scheduled.

Intervention

Participants randomized to the CDSMP arm were invited to attend a 6-wk, classroom-based program for diabetes self-management. The effectiveness of the CDSMP has been described elsewhere[10]. With the goal of increasing self-efficacy to ultimately decrease chronic disease related symptoms and avoidable healthcare utilization, the CDSMP teaches participants techniques to facilitate enhanced decision making, action planning, and effective communication. CDSMP workshops were hosted in clinical environments and community-based settings. While fidelity to the individual classes was not monitored, CDSMP license requires that lay leaders use pre-scripted materials and that experienced master trainers/lay leaders (who attend a required four-day training program) lead the workshops.

Participants randomized to the control arm did not receive any treatment other than their usual clinical diabetes care, along with some publicly available Texas Diabetes Council patient education materials.

Data collection

Study measures were obtained at orientation/baseline, 6 mo, and 12 mo of follow-up. Participants received monetary compensation in the form of a gift card for travel expenses and time, consisting of $20 at orientation and at the 12-mo follow-up visit.

At orientation, a questionnaire was administered to obtain several pieces of information including: (1) demographics such as age, gender, and race/ethnicity; (2) diabetes self-care activity monitoring (number of days, 0-7, that any specific self-care activity was performed in the past week) as measured by the Summary of Diabetes Self-Care Activities instrument; (3) self-reported health-related quality of life (HRQOL) measures (*e.g.*, number of days physical/mental health was not good); and (4) pain and fatigue measures (on a scale of 1-10, 1 indicating none and 10 severe). Questionnaires were administered every 6 mo. However, as our primary end point was 12 mo, analyses were only conducted for this time period.

Anthropometric data were obtained at orientation and at subsequent follow-up visits. Height in inches was measured without shoes. Weight was measured in pounds on a balance beam scale or an electronic scale without shoes. Body mass index (BMI) was computed from height and weight measurements. Blood pressures were recorded with either a mercury sphygmomanometer or a validated automated device. Participants who were unable to come in for their follow-up appointments had their height, weight, and blood pressure data abstracted from electronic health records (EHRs). Measures recorded fell within the range of 10 d prior to and 45 d after participants' scheduled follow-up dates. This was done to obtain participant visits as close to their target dates as possible, but also allow for enough time after the target date to accommodate for scheduling errors (*i.e.*, missed appointments, rescheduling).

Measures of HbA1c were collected from EHRs dating back 6 mo prior to orientation to the last day of study participation (45 d after the 12-mo follow-up period). If a participant did not have any HbA1c value within the EHR for any particular follow-up visit, a lab test was scheduled to obtain a measure. Of the HbA1c collected 6 mo prior to orientation, the value measured closest to the orientation date was considered as the baseline HbA1c value. HbA1c values that were measured on dates preceding the baseline HbA1c were not included; *i.e.*, HbA1c values included in the analysis were those collected since the baseline HbA1c and until the last day of study participation.

Definition of a completed follow-up participation

A participant was considered to have completed a follow-up if there was an available HbA1c within the designated follow-up period, *i.e.*, within the cut-off dates, defined as within 45 d after the scheduled follow-up dates. For the 6-mo follow-up measure, if at least one HbA1c was available after baseline and before the 6-mo cut-off, the participant was considered to have completed a follow-up. For the 12-mo follow-up measure, the designated range was between the 6-mo cut-off date and the 12-mo cut-off date. Participants who were unable to complete an assessment at one time period were not excluded from future assessments. For instance, if a participant did not have any HbA1c measured within the specified time period for their 6-mo follow-up but had one available for their 12-mo follow-up, he/she was considered to have completed the 12-mo follow-up, but not the 6-mo.

Outcome measures

The primary study outcome measure was change in HbA1c from randomization to 12 mo of follow-up. Secondary outcome measures included BMI and blood pressure, along with several self-management behavioral measures (*e.g.*, foot care) from randomization to 12 mo of follow-up.

Statistical analysis

Analysis was based on intent-to-treat. Descriptive statistics were used to describe baseline demographic, anthropometric, and clinical characteristics by study arm. Analysis of variance as used to compare average changes in self-management behaviors between study arms. To determine whether the treatment had an effect on the rate of change in HbA1c level over time, we used linear mixed models that included time as a continuous variable. A spatial power covariance structure with time as the distance measure accounted for the time-series correlation among repeated measurements on each subject. Forward selection was utilized, in which powers of time were added one at a time to the base model including treatment group effects only. Time and treatment effects were then added gradually and evaluated with likelihood ratio tests to assess any effect modification. The final mixed model included time, time squared, treatment group, and the interaction between time and treatment group as fixed effects. HbA1c values included in the analysis were those falling within the time frame of 6 mo prior to orientation until the 12-mo follow-up cut-off point.

RESULTS

Subject enrollment, participation and retention

The flow diagram of participant enrollment and disposition in the trial has been described elsewhere[31]. Of the subjects randomized, 101 entered the CDSMP arm and 95 entered the control arm. Of the participants assigned to the CDSMP, 75.6% attended 4 of 6 sessions required for successful completion.

Demographic data and baseline comparison of study population

Demographic and baseline clinical characteristics were generally comparable between the two groups (Table 1). The mean age of participants was 57.6 ± 10.9 years. Slightly more than a third (36.4%) was of minority status, self-reporting as either African American or Hispanic. The majority of participants had received post-secondary education; 40% had attended some college or vocational school, 20% were college graduates, and 13% had completed higher forms of education. Approximately one-third reported annual incomes greater than $50000, while almost 40% reported annual incomes between $25000 and $49999.

An overwhelming majority (92.9%) of the participants were either overweight or obese, with a mean BMI of 34.3 ± 7.4 kg/m^2. While measures of systolic blood pressure were comparable between study arms, with a mean of 134.8 ± 19.3 mmHg, measures of diastolic blood pressure were significantly different ($P < 0.002$). The mean baseline HbA1c for participants was 9.3% ± 1.6% and did not differ significantly between the two groups.

Table 2 summarizes participants' diabetes self-care activity (DSCA) monitoring, HRQOL measures, and pain and fatigue measures at baseline. Participants in the control arm reported checking their feet more frequently than those in the CDSMP arm ($P = 0.04$). Although participants in the control group reported inspecting the inside of their shoes more frequently and also tended to report fewer unhealthy physical days and experience less limited days due to physical and mental health, these did not reach statistical significance ($P \geqslant 0.05$).

Changes in HbA1c from baseline to 12 mo

There were modest but statistically significant reductions in HbA1c from baseline to 12 mo of follow-up. The results of the linear mixed model are presented in Table 3. The adjusted reductions in HbA1c over the 12 mo of follow-up for the CDSMP and control groups were 0.559% and 0.576%, respectively ($P < 0.0001$). However, the interaction term of the treatment group and time was not statistically significant ($P = 0.885$), implying no significant difference in HbA1c reductions by treatment assignment.

Changes in DSCA monitoring, HRQOL measures, and pain and fatigue measures

The mean difference in the number of days (within the last 7 d), from baseline to 12 mo of follow-up, that participants reported using specific diabetes self-care activity features were compared between the CDSMP and control arms (table not shown). While there were no differences on 12 of the 14 self-care indicators, participants in the control arm had a higher rate of change in checking their feet than those in the CDSMP arm (increase of 0.28 d/mo *vs* 0.20 d/mo; $P = 0.02$). Similarly, participants in the control arm reported an increase of 0.15 d/mo eat-

Table 1 Characteristics of study participants (n = 196)

	Controls (n = 95)		CDSMP (n = 101)		*P*-value
	No.	%	No.	%	
Age group (yr)					0.32
30-44	15	15.8	12	11.9	
45-64	55	57.9	69	68.3	
⩾ 65	25	26.3	20	19.8	
Gender					0.74
Female	53	55.8	54	53.5	
Male	42	44.2	47	46.5	
Hispanic					0.46
Yes	15	15.8	20	19.8	
No	80	84.2	81	80.2	
Minority[1]					0.32
Yes	32	33.7	41	40.6	
No	63	66.3	60	59.4	
Race/Ethnicity					0.60
African American	17	17.9	21	20.8	
Hispanic	15	15.8	20	19.8	
Neither Hispanic or African-American	63	66.3	60	59.4	
Income					0.40
< $15000	9	10.5	12	13.6	
$15000-$24999	16	18.6	11	12.5	
$25000-$49999	30	34.9	41	46.6	
$50000-$75000	17	19.8	12	13.6	
> $75000	14	16.3	12	13.6	
Education					0.48
High school graduate or less	25	26.3	26	25.7	
Some college/vocation school	36	37.9	46	45.5	
College graduate or higher	34	35.8	29	28.7	
HbA1c (%), mean ± SD	9.2	1.6	9.4	1.7	0.48
SBP (mm/Hg), mean ± SD	132.9	21.7	131.9	14.1	0.73
DBP (mm/Hg), mean ± SD	75.8	13.6	79.4	9.8	0.05
BMI (kg/m^2), mean ± SD	33.9	7.7	33.5	8.0	0.70

[1]African American or Hispanic. CDSMP: Chronic Disease Self-Management Program; HbA1c: Hemoglobin A1c; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; BMI: Body mass index.

Table 2 Baseline diabetes self-care activities monitoring, health-related quality of life, pain and fatigue measures

Measure	Controls	CDSMP	*P*
Diabetes self-care activity monitoring (d/wk)			
30 min of any physical activity?	3.01	3.50	0.17
Daily exercise session?	2.23	2.53	0.40
Test your blood sugar?	4.22	4.38	0.70
Test sugar times provider recommends?	3.58	3.29	0.50
Check your feet?	5.20	4.41	0.04
Wash your feet?	6.58	6.36	0.29
Soak your feet?	1.73	1.21	0.14
Dry between your toes?	5.21	5.37	0.68
Inspect inside of shoes?	3.25	2.43	0.06
Follow a healthful eating plan?	3.80	3.92	0.71
Space carbohydrates evenly?	3.25	3.12	0.74
Eat ⩾ 5 fruit/vegetable servings?	3.80	3.44	0.30
Eat high-fat products (red meat, full-fat diary)?	3.63	3.63	0.98
Eat packaged or bakery goods?	2.05	2.16	0.71
Health related quality of life (d/mo)			
Physical health not good	3.98	5.96	0.07
Mental health not good	4.09	4.72	0.56
Physical/mental health hindered usual activities	1.82	3.65	0.05
Pain and fatigue measures (scale 1-10)			
Average daily pain in the past 2 wk	3.74	3.74	1.00
Average daily fatigue in the past 2 wk	4.41	4.54	0.72

CDSMP: Chronic Disease Self-Management Program.

Table 3 Results from the linear mixed models

	Controls (n = 95) Mean ± SE[1]	CDSMP (n = 101) Mean ± SE[1]	Difference between the two groups Mean ± SE[1]
Baseline	9.018 ± 0.153	9.175 ± 0.149	0.157 ± 0.213
12 mo	8.442 ± 0.160	8.615 ± 0.156	0.173 ± 0.218
12 mo-Baseline	-0.576 ± 0.093[a]	-0.559 ± 0.091[a]	0.016 ± 0.112

[1]Adjusted means from linear mixed models. [a]P < 0.0001 for test *vs* H$_0$: mean equals to 0.

ing 5 or more servings of fruits and vegetables compared to an increase of 0.01 d/mo reported by those in the CDSMP arm (P = 0.02).

DISCUSSION

In this study, we sought to assess the effectiveness of the CDSMP on HbA1c and selected self-reported measures among patients with type 2 diabetes who were out of control. We found no significant differences between the CDSMP intervention and usual care in this integrated healthcare system. To the best of our knowledge, this is the first study to evaluate the effectiveness of the CDSMP in a randomized controlled trial in the United

States. It is also one of the first studies to evaluate and compare these interventions in a racially/ethnically diverse population in a practice setting outside of testing done by the original program developers. It therefore provides important exploratory data, shaping our knowledge and understanding of factors which may be important to minority and ethnic populations in adopting diabetes self-management techniques.

Our results corroborate the findings of others that participation in the CDSMP may be associated with better glycemic control[28]. However, a comparison with the control group indicates that usual care might do equally well. Therefore, our study findings need to be tempered due to the possibility of methodological confounds such as unaccounted group demographic and health differences at baseline, relatively small sample sizes, and better awareness among those in a clinical trial or high quality routine diabetes care that emphasizes the importance of glycemic control. For example, participants in this study were, on average, younger than those studied in other recent CDSMP studies[23,29]. Additionally, the controls in this study appeared slightly healthier and better educated than their counterparts in the CDSMP intervention which might have made them more receptive to both clinical and community-based diabetes self-management and obesity prevention messages. It should be noted that Scott and White Health System employs diabetes educators for their patients with diabetes. Scott and White also employs dedicated endocrinologists and their usual care for diabetes exceeds the recommendations set by the Texas Diabetes Association.

Other study limitations need to be noted. First, our subjects were selected from a randomized controlled trial with three interventions, restricting the numbers available in any one group. Second, post-hoc analysis showed that we were somewhat under-powered: we only had 60% power to detect a difference of 0.5% HbA1c reduction between the two groups at the current sample size. Other future analyses should focus on randomizing a larger number of participants in the treatment arm being investigated. Third, there were notable differences between the intervention and control groups, with the control group appearing to be healthier at baseline. Fourth, there was attrition in terms of treatment completion for the intervention group (75.6% attended 4 of 6 sessions required for successful completion) as well as differential research attrition between the two groups (14.9% or 15% participants in the treatment group and 23.2% or 22% participants in the control group did not have 12 mo data). Finally, this study was conducted in only one integrated health care system, limiting generalizability to other settings and populations.

There is also a debate in the self-management field regarding whether generic *vs* disease-specific self-management is more beneficial[24,32]. While our view was that a generic program would be valuable for patients experiencing several comorbidities including diabetes, more positive results might have been observed if the diabetes specific CDSMP was utilized (which was not evidence-based at the time of initial program selection for English speaking patients)[33].

In conclusion, we found in this study that although a behavioral intervention such as the CDSMP can result in some modest improvements in glycemic control, the same improvements may be found among participants that receive usual care. The reduction in HbA1c levels found in our control group that received usual care suggests that good routine care in an integrated healthcare system can also lead to better glycemic control. More research is needed to understand the benefits of self-management programs both independently and in conjunction with primary care. For example, are there settings where self-management programs might be especially needed, *e.g.*, in medically underserved areas? What kinds of participants might improve most with self-management programs? Such knowledge is important for providing better tailoring diabetes care to patients.

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COMMENTS

Background

The Stanford Chronic Disease Self-Management Program (CDSMP) represents one of the most studied evidence-based behavioral or self-care programs for chronic diseases including diabetes.

Research frontiers

The CDSMP has been found to be highly effective in improving the general health of people with several chronic conditions such as heart disease and arthritis. Recent evidence indicates that the CDSMP is a useful and appropriate program for lowering glycated hemoglobin A1c (HbA1c) among people with type 2 diabetes who are out of control.

Innovations and breakthroughs

This study demonstrated that the CDSMP may not lower HbA1c among people with type 2 diabetes any better than good routine care in an integrated healthcare system.

Applications

Findings from this study show that people with type 2 diabetes managed with good routine care in an integrated healthcare system can also have good glycemic control. Nonetheless more research is needed to understand the benefits of self-care programs in primary care.

Peer review

The study by Forjuoh *et al* aimed to assess the effectiveness of the CDSMP on the metabolic control. This is an interesting investigation from a practical point of view.

REFERENCES

1 **World Health Organization**. Diabetes. Geneva: World Health Organization, 2012 (Accessed on 27th November 2013). Available from: URL: http: //www.who.int/media-centre/factsheets/fs312/en/index.html

2 **Brod M**, Wolden M, Groleau D, Bushnell DM. Understanding the economic, daily functioning, and diabetes management burden of non-severe nocturnal hypoglycemic events in Canada: differences between type 1 and type 2. *J Med Econ* 2014; **17**: 11-20 [PMID: 24199622]

3 **Zhuo X**, Zhang P, Hoerger TJ. Lifetime direct medical costs of treating type 2 diabetes and diabetic complications. *Am J*

Prev Med 2013; **45**: 253-261 [PMID: 23953350 DOI: 10.1016/j.amepre.2013.04.017]
4 **Caprio S**. Development of type 2 diabetes mellitus in the obese adolescent: a growing challenge. *Endocr Pract* 2013; **18**: 791-795 [PMID: 23047931 DOI: 10.4158/EP12142.RA]
5 **Franek J**. Self-management support interventions for persons with chronic disease: an evidence-based analysis. *Ont Health Technol Assess Ser* 2013; **13**: 1-60 [PMID: 24194800]
6 **Health Quality Ontario**. Behavioural interventions for type 2 diabetes: an evidence-based analysis. *Ont Health Technol Assess Ser* 2009; **9**: 1-45 [PMID: 23074526]
7 **American Diabetes Association**. Standards of medical care in diabetes--2011. *Diabetes Care* 2011; **34** Suppl 1: S11-S61 [PMID: 21193625 DOI: 10.2337/dc11-S011]
8 **Funnell MM**, Brown TL, Childs BP, Haas LB, Hosey GM, Jensen B, Maryniuk M, Peyrot M, Piette JD, Reader D, Siminerio LM, Weinger K, Weiss MA. National Standards for diabetes self-management education. *Diabetes Care* 2011; **34** Suppl 1: S89-S96 [PMID: 21193633 DOI: 10.2337/dc11-S089]
9 **Handelsman Y**, Mechanick JI, Blonde L, Grunberger G, Bloomgarden ZT, Bray GA, Dagogo-Jack S, Davidson JA, Einhorn D, Ganda O, Garber AJ, Hirsch IB, Horton ES, Ismail-Beigi F, Jellinger PS, Jones KL, Jovanovič L, Lebovitz H, Levy P, Moghissi ES, Orzeck EA, Vinik AI, Wyne KL. American Association of Clinical Endocrinologists Medical Guidelines for Clinical Practice for developing a diabetes mellitus comprehensive care plan. *Endocr Pract* 2011; **17** Suppl 2: 1-53 [PMID: 21474420 DOI: 10.4158/EP.17.S2.1]
10 **Lorig KR**, Sobel DS, Stewart AL, Brown BW, Bandura A, Ritter P, Gonzalez VM, Laurent DD, Holman HR. Evidence suggesting that a chronic disease self-management program can improve health status while reducing hospitalization: a randomized trial. *Med Care* 1999; **37**: 5-14 [PMID: 10413387 DOI: 10.1097/00005650-199901000-00003]
11 **Lorig KR**, Ritter P, Stewart AL, Sobel DS, Brown BW, Bandura A, Gonzalez VM, Laurent DD, Holman HR. Chronic disease self-management program: 2-year health status and health care utilization outcomes. *Med Care* 2001; **39**: 1217-1223 [PMID: 11606875 DOI: 10.1097/00005650-200111000-00008]
12 **Foster G**, Taylor SJ, Eldridge SE, Ramsay J, Griffiths CJ. Self-management education programmes by lay leaders for people with chronic conditions. *Cochrane Database Syst Rev* 2007; **17**: CD005108 [PMID: 17943839]
13 **Brady TJ**, Murphy L, O'Colmain BJ, Beauchesne D, Daniels B, Greenberg M, House M, Chervin D. A meta-analysis of health status, health behaviors, and healthcare utilization outcomes of the Chronic Disease Self-Management Program. *Prev Chronic Dis* 2013; **10**: 120112 [PMID: 23327828 DOI: 10.5888/pcd10.120112.]
14 **Harrison M**, Reeves D, Harkness E, Valderas J, Kennedy A, Rogers A, Hann M, Bower P. A secondary analysis of the moderating effects of depression and multimorbidity on the effectiveness of a chronic disease self-management programme. *Patient Educ Couns* 2012; **87**: 67-73 [PMID: 21767927 DOI: 10.1016/j.pec.2011.06.007]
15 **Guilcher SJ**, Bereket T, Voth J, Haroun VA, Jaglal SB. Spanning boundaries into remote communities: an exploration of experiences with telehealth chronic disease self-management programs in rural northern ontario, Canada. *Telemed J E Health* 2013; **19**: 904-909 [PMID: 24134184]
16 **Griffiths C**, Motlib J, Azad A, Ramsay J, Eldridge S, Feder G, Khanam R, Munni R, Garrett M, Turner A, Barlow J. Randomised controlled trial of a lay-led self-management programme for Bangladeshi patients with chronic disease. *Br J Gen Pract* 2005; **55**: 831-837 [PMID: 16281998]
17 **Stone GR**, Packer TL. Evaluation of a rural chronic disease self-management program. *Rural Remote Health* 2010; **10**: 1203 [PMID: 20297869]
18 **Dongbo F**, Ding Y, McGowan P, Fu H. Qualitative evaluation of Chronic Disease Self Management Program (CDSMP) in Shanghai. *Patient Educ Couns* 2006; **61**: 389-396 [PMID: 15975756 DOI: 10.1016/j.pec.2005.05.002]
19 **Chan WL**, Hui E, Chan C, Cheung D, Wong S, Wong R, Li S, Woo J. Evaluation of chronic disease self-management programme (CDSMP) for older adults in Hong Kong. *J Nutr Health Aging* 2011; **15**: 209-214 [PMID: 21369669 DOI: 10.1007/s12603-010-0257-9]
20 **Elzen H**, Slaets JP, Snijders TA, Steverink N. Evaluation of the chronic disease self-management program (CDSMP) among chronically ill older people in the Netherlands. *Soc Sci Med* 2007; **64**: 1832-1841 [PMID: 17355901]
21 **Lorig K**, Ritter PL, Plant K. A disease-specific self-help program compared with a generalized chronic disease self-help program for arthritis patients. *Arthritis Rheum* 2005; **53**: 950-957 [PMID: 16342084 DOI: 10.1002/art.21604]
22 **Smith ML**, Ory MG, Ahn S, and Miles TP. Factors Associated with Women's Chronic Disease Management: Associations of Healthcare Frustrations, Physician Support, and Self-Care Needs. *J Aging Res* 2013; **2013**: 982052 [PMID: 24224090 DOI: 10.1155/2013/982052]
23 **Ory MG**, Ahn S, Jiang L, Smith ML, Ritter PL, Whitelaw N, Lorig K. Successes of a national study of the Chronic Disease Self-Management Program: meeting the triple aim of health care reform. *Med Care* 2013; **51**: 992-998 [PMID: 24113813 DOI: 10.1097/MLR.0b013e3182a95dd1]
24 **Brady TJ**, Murphy L. Sorting through the evidence for the arthritis self-management program and the chronic disease self-management program: Executive summary of ASMP/CDSMP meta-analysis [Internet]. 2011 (Accessed on 2nd December 2013). Available from: URL: http: //www.cdc.gov/arthritis/docs/ASMP-executive-summary.pdf
25 **Barlow JH**, Wright CC, Turner AP, Bancroft GV. A 12-month follow-up study of self-management training for people with chronic disease: are changes maintained over time? *Br J Health Psychol* 2005; **10**: 589-599 [PMID: 16238867 DOI: 10.1348/135910705X26317]
26 **Kennedy A**, Reeves D, Bower P, Lee V, Middleton E, Richardson G, Gardner C, Gately C, Rogers A. The effectiveness and cost effectiveness of a national lay-led self care support programme for patients with long-term conditions: a pragmatic randomised controlled trial. *J Epidemiol Community Health* 2007; **61**: 254-261 [PMID: 17325405 DOI: 10.1136/jech.2006.053538]
27 **Goeppinger J**, Armstrong B, Schwartz T, Ensley D, Brady TJ. Self-management education for persons with arthritis: Managing comorbidity and eliminating health disparities. *Arthritis Rheum* 2007; **57**: 1081-1088 [PMID: 17665471 DOI: 10.1002/art.22896]
28 **Lorig K**, Ritter PL, Ory MG, Whitelaw N. Effectiveness of a generic chronic disease self-management program for people with type 2 diabetes: a translation study. *Diabetes Educ* 2013; **39**: 655-663 [PMID: 23782621 DOI: 10.1177/0145721713492567]
29 **Ory MG**, Ahn S, Jiang L, Lorig K, Ritter P, Laurent DD, Whitelaw N, Smith ML. National study of chronic disease self-management: six-month outcome findings. *J Aging Health* 2013; **25**: 1258-1274 [PMID: 24029414 DOI: 10.1177/0898264313502531]
30 **Altman DG**, Schulz KF, Moher D, Egger M, Davidoff F, Elbourne D, Gøtzsche PC, Lang T; CONSORT GROUP (Consolidated Standards of Reporting Trials). The revised CONSORT statement for reporting randomized trials: explanation and elaboration. *Ann Intern Med* 2001; **134**: 663-694 [PMID: 11304107 DOI: 10.7326/0003-4819-134-8-200104170-00012]
31 **Forjuoh SN**, Bolin JN, Huber JC, Vuong AM, Adepoju OE, Helduser JW, Begaye DS, Robertson A, Moudouni DM, Bonner TJ, McLeroy KR, Ory MG. Behavioral and technological interventions targeting glycemic control in a racially/ethnically diverse population: a randomized controlled trial. *BMC Public Health* 2014; **14**: 71 [PMID: 24450992 DOI:

10.1186/1471-2458-14-71]
32 **Sevick MA**, Trauth JM, Ling BS, Anderson RT, Piatt GA, Kilbourne AM, Goodman RM. Patients with Complex Chronic Diseases: perspectives on supporting self-management. *J Gen Intern Med* 2007; **22** Suppl 3: 438-444 [PMID: 18026814 DOI: 10.1007/s11606-007-0316-z]
33 **Lorig K**, Ritter PL, Villa F, Piette JD. Spanish diabetes self-management with and without automated telephone reinforcement: two randomized trials. *Diabetes Care* 2008; **31**: 408-414 [PMID: 18096810 DOI: 10.2337/dc07-1313]

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In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

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No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001

Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 200201 03498. 2002 Aug 1

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